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DETERMINATION OF NITROSAMINES IN INDUSTRIAL AND MUNICIPAL WASTEWATERS

by

John W. Rhoades
Richard E. Thomas
Donald E. Johnson
Southwest Research Institute
San Antonio, Texas 78284

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Project Officer

Mr. James E. Longbottom, Project Officer
Environmental Monitoring and Support Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
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The procedural steps of sample preservation, extraction, cleanup of extracts, and gas chromatographic separation and detection in the analysis of three N-nitrosamines were studied. Two gas chromatographic columns and three detector systems, alkali flame ionization, thermal energy analyzer, and Hall electrolytic conductivity, were evaluated. Extractions were performed with dichloromethane forom NaCl saturated and nonsalted samples at three pH's to determine extraction efficiencies. The effects of storage for seven days in the presence of residual chlorine and at two temperatures were determined. Florisil and alumina were compared as adsorbents for the cleanup of extracts. Recoveries of the substances for clean water and wastewater were measured, and assessments of accuracy and precision were made.

The method is satisfactory for use with AFID for the analysis of clean water and wastewater having a relatively low background of interferences. With TEA and Hall detection, samples can be analyzed for nitrosodimethylamine and nitrosodipropylamine with the sample cleanup. Nitrosodiphenylamine must be separated from diphenylamine prior to determination by AFID.

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FOREWORD

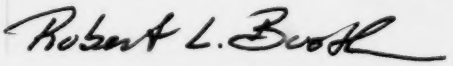
Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory-Cincinnati conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.

- Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water. Conduct studies to determine the responses of aquatic organisms to water quality.

- Conduct an agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

Under provisions of the Clean Water Act, the Environmental Protection Agency is required to promulgate guidelines establishing test procedures for the analysis of pollutants. The Clean Water Act Amendments of 1977 emphasize the control of toxic pollutants and declare the 65 "priority pollutants" and classes of pollutants to be toxic under Section 307(a) of the Act. This report is one of a series that investigates the analytical behavior of selected priority pollutants and suggests a suitable test procedure for their measurement.



Robert L. Booth, Acting Director
Environmental Monitoring and Support
Laboratory - Cincinnati

ABSTRACT

The procedural steps of sample preservation, extraction, cleanup of extracts, and gas chromatographic separation and detection in the analysis of three N-nitrosamines were studied. Two gas chromatographic columns and three detector systems, alkali flame ionization, thermal energy analyzer, and Hall electrolytic conductivity, were evaluated. Extractions were performed with dichloromethane from NaCl saturated and nonsalted samples at three pH's to determine extraction efficiencies. The effects of storage for seven days in the presence of residual chlorine and at two temperatures were determined. Florisil and alumina were compared as adsorbents for the cleanup of extracts. Recoveries of the substances for clean water and wastewater were measured, and assessments of accuracy and precision were made.

The method is satisfactory for use with AFID for the analysis of clean water and wastewater having a relatively low background of interferences. With TEA and Hall detection, samples can be analyzed for nitrosodimethylamine and nitrosodipropylamine with no sample cleanup. Nitrosodiphenylamine must be separated from diphenylamine prior to determination by AFID.

This report was submitted in partial fulfillment of Contract 68-03-2606 by Southwest Research Institute under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from September 1977 to April 1979 when work was completed on the developmental phase of the contract.

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SECTION 1

INTRODUCTION

This project on nitrosamines was part of an overall program of the Environmental Protection Agency to establish test procedures for the analysis of 114 toxic substances in wastewaters. These toxic compounds have been separated into twelve categories as follows:

Category	Compound
1	Phthalate Esters
2	Haloethers
3	Chlorinated Hydrocarbons
4	Nitrobenzenes and Isophorone
5	Nitrosamines
6	Dioxin
7	Benzidines
8	Phenols
9	Polynuclear Aromatics
10	Pesticides and PCBs
11	Purgeables
12	Acrolein, Acrylonitrile, Dichlorodifluoromethane

This is the report on Category 5: Nitrosamines.

The specific nitrosamine compounds studied in this effort were:

1. Nitrosodimethylamine (NDMA)
2. Nitrosodipropylamine (NDPrA)
3. Nitrosodiphenylamine (NDPhA)

It was desirable that where possible common sample treatment for the various categories be employed in order to minimize the cost of analyzing unrelated compounds in any given water sample. The efforts reported under the following performance headings were designed to provide information relative to this common purpose.

The study was conducted in two phases. In Phase I work was conducted with clean water and was intended to provide information which would give direction to Phase II work, conducted on actual wastewaters, and serve as a comparison base for the information developed.

The ultimate objective was to develop a method having a maximum of applicability, which could be published in the Federal Register and carried out in most analytical laboratories. Other objectives included the development of accuracy and precision information for the various steps in the method and for procedure variations as required by a particular wastewater, as well as to maximize the use of common sample treatment steps for all of the categories of organic toxic substances listed above.

SECTION 2

CONCLUSIONS

Nitrosodimethylamine (NDMA) and nitrosodipropylamine (NDPrA) can be gas chromatographed very well. Nitrosodiphenylamine (NDPhA) is thermally degraded in the injector, apparently producing mainly NO and diphenylamine (DPhA). Nitrosodiphenylamine and diphenylamine when injected produce peaks with the same retention time, and consequently these two compounds must be separated prior to gas chromatographic analyses for nitrosodiphenylamine by detection of the thermally produced diphenylamine.

Extraction of NDPrA and NDPhA with dichloromethane (DCM) from salted or nonsalted clean water was good, averaging about 98 percent and 80 percent respectively. NDMA is extracted from clean water at about 40 percent efficiency when not salted and about 68 percent efficiency when salted. The overall benefit of salting does not appear to be sufficient to warrant salting as a routine step in the extraction procedure. The pH in the range of 2-10 had essentially no effect on the extraction efficiency of any of the three nitrosamines under study.

The nitrosamines can be determined at concentrations of a few tenths of a µg/L for both clean water and wastewaters with each detection system tested with the exception of the nitrosodiphenylamine, which cannot be determined by the thermal energy analyzer (TEA).

Alumina and Florisil column chromatography proved helpful as a cleanup procedure for AFID determinations of NDMA and NDPrA. The column chromatography also afforded a means of separating NDPhA from the DPhA. The DPhA could not satisfactorily be extracted from the DCM extract by an aqueous acid as could other amines.

Preservation studies with clean water indicated pH, temperature, and 2 ppm Cl_2 had little effect, if any, on NDMA and NDPrA during storage for seven days. All of the above factors produced some effect on storage life of NDPhA. Storage at 4°C at a pH slightly alkaline and preferably in the absence of residual chlorine were the best storage conditions.

Extraction recovery and preservation studies with wastewaters indicate possible losses, especially with NDPhA, with some wastewaters.

In summary, the extraction and cleanup methods developed are satisfactory for use with both clean water and wastewater, although the stability of some of the nitrosamines in wastewater may be poor even under the best storage conditions.

SECTION 3

RECOMMENDATIONS

The primary gas chromatography column used in this work, which was composed of 10 percent Carbowax 20M + 2 percent KOH on 80/100 mesh Chromosorb WAW, is preferred over other column packings because of the delayed elution time of NDMA relative to the solvent peak. It is necessary to chromatograph the NDPhA at a higher temperature of 220°C than the NDMA and NDPrA that were chromatographed at 110°C. Although other columns might be somewhat preferred for only the NDPhA determination alone, a single column at two different isothermal temperatures is preferred over two different columns. Therefore, even though the NDPhA has a somewhat longer elution time than desired, it is probably the best compromise column and it performed well during the program work.

Several factors must be considered in deciding which detector system would be most appropriate for determination of the three nitrosamines in wastewaters. There is little doubt that the TEA and the Hall in the pyrolytic mode are more selective for NDMA and NDPrA than the AFID. The TEA is not capable of detecting the DPhA produced from NDPhA at injection. The Hall detector in the pyrolytic mode also does not detect the DPhA but will detect the DPhA in the hydrogenation mode. The Hall in the hydrogenation mode will also detect NDMA and NDPrA but with no more selectivity than the AFID. The AFID is easier to operate and maintain than the Hall. Considering these facts, the AFID is probably the detector of choice when detection of all three nitrosamines is required and interferences from the constituents of the wastewater are not excessive. Since this was the case, the AFID was the detector of choice.

Although NDMA extraction is improved when the water sample is saturated with NaCl, the overall benefits are not sufficient to recommend the addition of salt as a routine part of the extraction procedure.

Water samples should be stored at 4°C and pH 7-10 in the dark. Samples can be extracted upon removal from storage without further alteration, although it should be verified if the pH is still basic.

Due to the complexities of the method, only chemists experienced with the method or persons under their close supervision should conduct analyses for these nitrosamines.

It should be realized that the applicability of the method can be severely limited by interferences which cannot be removed and that verification by other detectors and/or mass spectrometry may be required with some samples.

SECTION 4

PHASE I

OBJECTIVES

The objectives of Phase I were limited to:

1. review of scientific literature for key references relative to determination of N-nitroso compounds in water and other matrices;
2. determination of the best gas chromatographic columns and conditions for detection of N-nitroso compounds when using the detectors of interest;
3. determination of extraction efficiency for N-nitroso compounds from clean water samples;
4. determination of the effects on the substances of storage for seven days at two temperatures, three pH levels, and with or without free chlorine in the water.

PROGRAM DIRECTION

Before experimental work began, representatives of SwRI met in Cincinnati with the project officer and others of concern in the EPA Cincinnati laboratories to discuss and decide on certain specific aspects of the activities in Phase I. In addition to the decisions reached on GC columns, extraction solvents, and other experimental details which will be given in subsequent sections of this report, guidelines were developed as follows:

- Unusual, exotic, or overly expensive items are not desirable. Simplicity is to be preferred wherever the option exists. For example, mass spectrometry and WCOT or SCOT columns are ruled out.
- Use of chemical treatment to produce a derivative which cannot be unequivocally related to the parent substance is not acceptable.
- Quantitation by electronic integration of peak area or by peak height measurement is acceptable.

EXPERIMENTAL

Literature Search

A comprehensive literature search was made using a terminal linked to Lockheed Information Systems' DIALOG®. Chemical Abstract Condensates (CAC) were searched for the years 1972 through the present. Also, NTIS (1964 to present), Enviroline (1971 to present), and Pollution Abstracts (1970 to present) were searched. In some cases, both on-line and off-line prints were obtained, the former in order to rapidly obtain a small enriched set of references to work with and the latter to provide comprehensive coverage by retrieval of a larger set of references.

The literature search as conducted yielded 200 to 300 references. Examination of titles and in many cases abstracts reduced this to about 50 references which are listed in Appendix A.

The International Agency for Research on Cancer has conducted many Working Conferences on N-Nitroso Compounds. The proceedings of these conferences are available in the form of IARC Scientific Publications from the World Health Organization (WHO). The title of each publication is listed below since the series of publications is considered to be the best single source of a wide variety of information on N-nitroso compounds:

N-Nitroso Compounds--Analysis and Formation. Proceedings held at Heidelberg Germany, October 1971, IARC Scientific Publication 3

N-Nitroso Compounds in the Environment. Proceedings of Working Conference Held at Lyon, France, October 1973, IARC Scientific Publication 9

Environmental N-Nitroso Compounds--Analysis and Formation. Proceedings of Working Conference held at Tallinn, Estonia, IARC Scientific Publication 14

Fifth Meeting on Analysis and Formation of N-Nitroso Compounds. Proceedings of Working Conference held at Durham, New Hamphsire, 1977, IARC Scientific Publication 19

Prior to 1973, most of the analytical work on N-nitroso compounds was directed towards the analysis of food products. Detectors such as the AFID, Coulson Electrolytic Conductivity Detector (CECD), Hall Electrolytic Conductivity Detector (HECD), or mass spectrometers were used to give selective detection.

With the introduction of the Thermal Energy Analyzer by the Thermo Electron Corporation, Dr. David H. Fine and his group at Thermo Electron Corporation have expanded analytical efforts into other environmental areas of interest such as air, water, cosmetics and pesticides. The work of Dr. Fine and his group over the last few years is therefore of particular interest and his publications, as are the IARC publications, are considered to be major contributions to the N-nitrosamine literature.

Many different gas chromatographic column packings have been used for N-nitrosamine analysis. Porous polymer beads, silicones, diethylene glycol succinate, various Carbowaxes, and FFAP have all been used successfully. Carbowax 20M has frequently been used and was recommended by SwRI as the column packing of choice.

Extraction of N-nitroso compounds from water is accomplished almost exclusively with dichloromethane (DCM) because of the greater extraction efficiency of this solvent, especially for NDMA.

For the analysis of volatile nitroso compounds in foods, distillation is generally required before the DCM extraction. As part of the cleanup procedure, base, generally K_2CO_3, is added to the sample before distillation to remove acidic material or K_2CO_3 is added to the distillate, which is then extracted with DCM. The DCM is then acid washed and upon concentration may be ready for GC analysis. This is the general scheme for analysis of N-nitroso compounds in foods, with minor variations being introduced by various investigators. Further cleanup, if necessary, may be accomplished by alumina or silica gel column chromatography.

Water analysis for volatile N-nitrosamines in theory is somewhat simpler than the analysis for these compounds in foods, primarily because a distillation step is not required. A simple DCM extraction with appropriate water pH adjustments may be all that is required, depending upon the selectivity of the GC detector. The TEA detector is much more selective than the AFID. The cleanup procedure required for analysis by AFID would therefore be adequate or unnecessary for samples to be analyzed by TEA. Fine has shown that for many water samples little or no cleanup is required when using the TEA. The cleanup requirements will therefore be dictated by the water "contaminants" seen by the AFID.

Little information was obtained in the literature search relative to preservation of collected water samples.

Nitrosamines are readily subject to photodegradation and must be stored protected from light. Fine et al. stored collected water samples from the Baltimore area in brown bottles at 4°C for an unspecified time, but presumably not for more than a few days.

Gas Chromatography

At the preliminary meeting, it was decided that columns of Carbowax 20M + 2 percent KOH on Chromosorb W and methyl silicone on Ultra-Bond would be examined. In addition, it was deemed desirable to examine two more column packings. These two additional column packings were chosen to have polarities, as indicated by McReynolds constants, between the nonpolar methyl silicone and the moderately polar Carbowax 20M.

The column packings examined were:

Column 1 10 percent Carbowax 20M + 2 percent KOH on 80/120 mesh Chromosorb WAW (purchased from Supelco, Inc., Catalog 1-1805).

Column 2 10 percent SP-2250 on 100/120 mesh Supelcoport (purchased from Supelco, Inc., Catalog 1-2132). This is a methyl phenyl silcone similar to OV-17.

Column 3 10 percent SP-2100 (Supelco, Inc.) on 100/120 mesh Ultra-Bond (RFR Corporation, Catalog RGC-002). The column packing was prepared at Southwest Research Institute.

Column 4 10 percent SP-2401 on 100/120 mesh Supelcoport (purchased from Supelco, Inc., Catalog 1-1979). This packing is similar to QF-1 and OV-210.

The McReynolds constants for these stationary phases, as shown in Supelco's catalog, are as follows:

Stationary phase	McReynolds Constants				
	X'	Y'	Z'	U'	S'
SP-2100	017	057	045	067	043
SP-2250	119	158	162	243	202
SP-2401	146	238	358	468	310
Carbowax 20M	322	536	368	572	510

For this part of the program, all standards were prepared using acetone as the solvent.

It was found that nitrosodiphenylamine cannot be directly gas chromatographed. It decomposes immediately in the injector giving NO and diphenylamine as the primary degradation products. The NO can be detected using the TEA and the diphenylamine can be detected using the AFID.

The initial study of gas chromatographic columns using the AFID was made with nitrosodimethylamine, nitrosodiethylamine and nitrosodiphenylamine. At about the same time the TEA was received, the EPA required that nitrosodipropylamine be substituted for the nitrosodiethylamine. The chromatographic column study with the AFID using nitrosodiethylamine was not redone; however, the chromatographic column study using the TEA and the sensitivity determinations for both the TEA and the AFID was performed with the NDPrA.

For the purpose of establishing the gas chromatographic characteristics of a column packing, it makes little difference whether nitrosodiethylamine or nitrosodipropylamine was used. Neither of these compounds is as polar as NDMA and they are separated from each other primarily on the basis of boiling point.

For column packing evaluations using the AFID, all-glass columns 1.8-m x 4-mm ID were used. All the SP phase columns were conditioned at 250°C for a minimum of 30 hours with flow. The Carbowax 20M column was conditioned at 230°C for 30 hours with flow. It was necessary to operate at two isothermal column temperatures, a relatively low column temperature for the NDMA and the nitrosodiethylamine (NDEA) and a higher column temperature for the diphenylamine (DPhA). The gas chromatograph used was a Perkin-Elmer Model 900 with a Perkin-Elmer nitrogen detector of the electrically heated bead type. The carrier gas flow was 40 mL per minute. The hydrogen flow was 5 mL per minute with an initial electric bead heat setting of 790. Column temperature and attenuation setting were adjusted to obtain informative chromatograms.

Using the TEA, gas chromatography of the same four column packing was examined. All-glass columns 180-cm x 2-mm ID were packed with the various column packings for this purpose.

All column packings examined performed satisfactorily when reasonably large amounts of NDMA were injected. With the silicone columns, the NDMA peak is not adequately separated from the solvent peak, at the more sensitive electrometer setting, when using the AFID.

Figures A-1 through A-8 in Appendix A are chromatograms of the nitrosamines on Column 1, consisting of 10 percent Carbowax 20M + 2 percent KOH, and on Column 2, consisting of 10 percent SP-2250, with the AFID and the TEA.

Column 1 was chosen as the best column to use for the remainder of the program and the sensitivity values were determined for this column packing.

The sensitivity of the AFID decreases as the bead ages. This loss in sensitivity can be compensated to some exent by increasing the bead current until such time as the noise becomes excessive. It is then necessary to exchange the bead. However, the sensitivity level of the AFID is somewhat of a variable depending upon the condition of the bead.

Table 1 shows the retention times and sensitivities obtained under the noted gas chromatographic conditions.

TABLE 1. GAS CHROMATOGRAPHY OF NITROSAMINES

Nitrosamine	Retention time (min.) Column 1	Column 2	AFID sensitivity, pg (10X noise level)	TEA sensitivity, pg (10X noise level)
N-nitrosodimethylamine	4.1	0.88	120	138
N-nitrosodi-n-propylamine	12.1	4.2	460	375
N-nitrosodiphenylamine	12.8*	6.4**	900	--

Column 1 conditions: Chromosorb WAW 80/100 mesh coated with 10% Carbowax 20M/2% KOH packed in a 180 cm long x 4 mm ID glass column with helium carrier gas at 40 mL/min flow rate. Column temperature is 110°C except where * indicates 220°C.

Column 2 conditions: Supelcoport 100/120 mesh coated with 10% SP-2250 packed in a 180 cm long x 4 mm ID glass column with helium carrier gas at 40 mL/min flow rate. Column temperature is 120°C except where ** indicates 210°C.

Extraction Studies

Dosing Solution--

An acetone dosing solution was prepared, based on sensitivity levels previously determined, such that 100 µL of the dosing solution contained the quantities of substances shown in Table 2.

TABLE 2. DOSING SOLUTION

Substance	Quantity per 100 µL (ng)
NDMA	400
NDPrA	1200
NDPhA	9000

When 100 µL was added to one liter of water, the concentrations would be 0.4, 1.2, and 9.0 µg/L for NDMA, NDPrA and NDPhA, respectively.

Solvent Effects--

The extraction studies were, of necessity, preceded by efforts to determine the best methods for final sample concentration which would provide samples compatible for use with the AFID. To eliminate or reduce the effects of DCM, it was necessary to add a nonhalogenated solvent during the final stages of the Kuderna-Danish evaporation to replace the DCM as the final solvent.

Acetone and hexane were chosen as the first solvents to study as "keeper" solvents during the DCM evaporation step. The TEA was used for this purpose so that recovery data for NDPhA could also be obtained with clean samples, since the NO liberated from the NDPhA in the injector results in an early eluting peak which is quantitatively detected by the TEA. The tests were conducted by dosing each of six 180-mL volumes of DCM with 100 µL of dosing solution. All six samples were evaporated down to about 10 mL at which time 20 mL of acetone was added to three samples and 20 mL of hexane was added to the other three samples. All six samples were evaporated to 5 to 7 mL and transferred to 10-mL volumetric flasks, each flask being made to volume with appropriate solvent, either acetone or hexane. The results of this evaporation study are shown below:

	% Recovered					
	Acetone Added			Hexane Added		
Compound	1	2	3	1	2	3
Nitrosodiphenylamine	89	85	87	3*	3*	3*
Nitrosodimethylamine	100	92	106	80	67	75
Nitrosodipropylamine	105	106	102	88	78	90

* Equal to solvent response

The results clearly show that acetone not only reduces loss of the lower boiling nitroso compounds, but also greatly reduces the thermal degradation of the NDPhA which was completely destroyed when hexane was used.

These same samples when analyzed using the AFID showed lower recoveries for the NDMA and the NDPrA. This apparent loss when using the AFID results from incomplete removal of the DCM, as determined by IR, which changes the AFID response and gives erroneous results. It was found that complete removal of DCM could not be accomplished even when as much as 100 mL of acetone was added to 10 mL of DCM and concentrated to 10 mL in a Kuderna-Danish evaporator.

A venting system was fabricated and placed in service on the gas chromatograph equipped with the AFID. By venting the solvent to the atmosphere, DCM contact with the rubidium bead and the problem of reduced sensitivity would be eliminated. Although considerable improvement was noted as a result of the venting system, the problem of residual DCM was still apparent at a reduced level.

Methanol was then examined as a replacement "keeper" solvent. Each of three Kuderna-Danish evaporators, containing 10 mL DCM and 20 mL methanol, was dosed with 100 µL of dosing solution and evaporated to a final volume of 10 mL in a 90°C water bath. The recoveries using methanol as "keeper" solvent were as follows:

	NDMA	NDPrA	NDPhA
TEA	98-93-98	100-95-100	91-89-94
AFID	94-98-98	96-99-98	

As can be seen, the recoveries for NDMA and NDPrA are close to 100 percent and the recovery for NDPhA was as good as for acetone. In addition, the AFID results are in good agreement with the TEA results, indicating little or no interference from the solvent. Thus, methanol was chosen as the "keeper" solvent for the extraction and preservation studies.

Extraction Procedures and Results--

The extraction studies were performed using Southwest Research Institute well water which was not chlorinated. The pH of water samples was adjusted by the addition of H_2SO_4 and NaOH as needed. A dosing solution was prepared which contained 0.4 µg NDMA, 1.2 µg NDPrA, and 9.0 µg NDPhA per 100 µL. Samples were extracted at pH 2, 7 and 10 with and without the addition of 300 g NaCl per liter of water.

The procedure used was as follows:

EXTRACTION PROCEDURE

1. One liter of test water (pH 2, 7, or 10) measured into one-liter Erlenmeyer.
2. NaCl (300 grams) added and dissolved if NaCl required.
3. Dosing solution (100 µL) injected into water and swirled to mix.
4. Dosed water sample quantitatively transferred to two-liter separatory funnel with Teflon stopcock.
5. DCM (60 mL) added to one-liter Erlenmeyer shaken and transferred to the two-liter separatory funnel.
6. Separatory funnel shaken continuously for two minutes.
7. After separating, the DCM layer (lower) drained into Kuderna-Danish evaporatory (500-mL capacity with 10 mL lower concentrator tube) through Na_2SO_4 to remove water.
8. DCM (60 mL) added to separatory funnel.
9. Repeat 6 and 7.
10. Repeat 8 and 9.
11. Kuderna-Danish evaporator (containing approximately 160 mL DCM) placed in 85-90°C water bath.

12. When DCM concentrated to 15-20 mL, 20 mL of MeOH added via condenser tube.

13. Evaporation continued until 6-8 mL MeOH remains.

14. Transferred to 10 mL volumetric flask and made to volume with MeOH.

15. Dosing solution (100 µL) injected into 10-mL volumetric flask (containing about 5-6 mL MeOH) and made to volume with MeOH. This is the "standard" and represents 100 percent recovery.

16. Sample (10 µL) injected onto the chromatographic column and the results compared to 10-µL injection of "standard."

All samples were analyzed by both AFID and TEA for NDMA and NDPrA. Recovery data for NDMA and NDPrA were based on peak height measurements. NDPhA results are based on NO liberated in the injector and electronic integration of peak area. NDPhA was not determined by AFID since it could not be determined in the presence of DPhA. Figure B-1 shows example chromatograms by both TEA and AFID of the standard and sample, pH 10-no salt.

The data are presented in Table 3 (TEA analysis) and Table 4 (AFID analysis).

Data Analysis-Extraction--

The results of the extraction study were submitted to statistical analysis to determine which of the factors affected the recovery efficiency of the method. The statistical model for these data is a two way analysis of variance (ANOVA) defined by:

$$Y_{ijk} = \mu + \tau_i + \rho_j + \tau\rho_{ij} + \varepsilon_{ijk}$$

where μ = overall mean

τ_i = effect of presence or absence of salt, i = 1, 2

ρ_j = effect of pH level j, j = 1, 2, 3

$\tau\rho_{ij}$ = interactive effect between salt level i and pH j

and ε_{ijk} = random error associated with observation Y_{ijk}.

Both salt and pH are considered fixed effects for this model. The five percent level is used on all tests of significance. Tables of the ANOVA's conducted are presented in Appendix C.

The results from each detector for each compound are considered separately in the analysis. For NDMA and NDPrA, the evaluation of the end results is made by comparing the results for both detectors for consistency.

TABLE 3. EXTRACTION RECOVERY (%) TEA

		NDMA		NDPrA		NDPhA	
		No Salt	Salt	No Salt	Salt	No Salt	Salt
pH 2	Blank	0	0	0	0	2	5
	1	42	66	97	96	84	81
	2	42	68	98	97	83	79
	3	40	68	98	97	83	78
	Avg.	41	67	98	97	83	79
pH 7	Blank	0	0	0	0	2	2
	1	38	66	95	99	89	82
	2	41	69	95	98	84	81
	3	42	66	98	101	87	80
	4	40	70	99	101	77	79
	Avg.	40	68	97	100	84	81
pH 10	Blank	0	0	0	0	3	3
	1	40	69	97	98	86	82
	2	42	65	98	97	87	77
	3	40	69	98	99	90	78
	Avg.	41	68	98	98	88	79

TABLE 4. EXTRACTION RECOVERY (%) AFID

		NDMA		NDPrA	
		No Salt	Salt	No Salt	Salt
pH 2	Blank	0	4	0	0
	1	43	68	98	98
	2	42	66	102	94
	3	43	69	99	99
	Avg.	43	68	100	97
pH 7	Blank	4	7	0	0
	1	38	75	96	95
	2	39	71	92	98
	3	41	70	91	95
	4	45	72	94	99
	Avg.	41	72	94	97
pH 10	Blank	5	5	0	0
	1	41	76	101	99
	2	42	74	99	98
	3	40	74	97	98
	Avg.	41	75	99	98

The summary of the ANOVA for NDMA results on the TEA analyzer indicates the only significant term in the model is the salt factor, where the addition of salt resulted in significantly higher average recoveries. The variation for pH levels and for interaction was less than the between replicate variation.

The means of the various factors are shown at the bottom of the table in Appendix C. The mean recovery for the samples with salt was 67.6 percent compared to 40.7 percent for the unsalted samples. This represents a recovery increase of about two-thirds relative to the unsalted samples.

For the samples run on the AFID, the results were similar. The factor of salt was extremely significant, with mean recoveries of 71.3 for the samples with salt and 41.4 for those with no salt. The pH-salt interaction term was significant for these data however and this was investigated further. To determine where the differences lie, a least significant difference (LSD) statistic was calculated, according to the formula

$$LSD_{0.05} = 2.145\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right) MS_E}$$

where n_1 = number of samples used to obtain mean 1

n_2 = number of samples used to obtain mean 2

MSE = mean square for error from the ANOVA

2.145 = t-value with 14 df at the 5-percent significance level.

This calculates to be 3.3 when comparing means based on samples of size 3 and 4. Using this statistic, no difference can be detected among the means where no salt was added. Where salt was added, the indication is that a lower recovery was obtained at pH 2 than with the other two pH's. It may be noted that the lowest average recovery for the TEA data was also at pH 2 for the samples with salt added, which gives some credence to the result. However, the magnitude of the difference in recoveries is rather small, and no major problem is indicated for the recovery efficiency dependent upon the pH of the sample.

The NDPrA results for the TEA detected no significant factors in the analysis, and the overall recovery was quite high, nearly 98 percent. Thus, these data indicate that there is no preference for adding or not adding salt, adjusting the pH, or any combination of the two. The same recovery can be expected regardless of conditions.

A significant pH factor was observed on the AFID results for NDPrA. Clearly, the data indicate that the results of the analyses at pH 7 are low relative to those at pH 2 and 10. This is not an explicable result, however, and the results of the TEA analyses do not support this conclusion. More importantly, the magnitude of the lowest average

recovery, 95.1 percent, is such that no problem is indicated for future uses of the method regardless of pH.

The statistical summary for the NDPhA analyses shows that salt was determined to be a significant factor. The samples without salt yielded a mean of 85 percent recovery versus 79.7 percent for the samples with salt added. On a relative basis, this represents about a seven percent increase. The recoveries under both sets of conditions were good, however. Neither the pH factor nor the interaction was a significant source of variation in the results, and no consideration of pH seems necessary in future analyses.

In summary, the most obvious result was the difference in recoveries for NDMA with the addition of salt, which averaged 27 percent on the TEA and 30 percent on the AFID. The recoveries were 41 percent and 68 percent for the TEA and 41 percent and 71 percent for the AFID for without and with salt, respectively. The addition of salt made no difference on the recovery of NDPrA, but did slightly lower the recoveries of NDPhA. The mean recoveries for this compound were 85 percent with no salt and 80 percent with salt added.

The pH of the water did not appear to be a cause of concern. The repeatability of the results was of such a consistent nature that it was possible to separate average recoveries which differed by only two percent recovery. In the case of the NDMA and NDPrA, it was concluded that both of the detectors should show the difference for the trend to be considered significant. As can be seen, this was not observed. For NDMA, the results from the two detectors were not consistent, with the combination of added salt and pH 10 showing a better result on the AFID, but no pH or interactive effect detected on the TEA. For NDPrA, pH 7 alternatively was the most and least favored of the pH's, indicating no real difference existed among pH's. These results are summarized in Table 5.

TABLE 5. SUMMARY OF EXTRACTION STUDY RESULTS

Compound	Detector	Salt	pH	Remarks
NDMA	TEA	Added	--	Recovery higher by 27% with salt.
	AFID	Added	10	Recovery 30% higher with salt; 2.3% increase under basic conditions.
NDPrA	TEA	--	--	Slightly better at pH 7 with salt.
	AFID	--	Basic	Slightly lower at pH 7.
NDPhA	TEA	None	--	Recovery 5% higher without salt.

Preservation Studies

The dosing and extraction techniques used in the extraction studies were used in the preservation studies. A fresh dosing solution in acetone was prepared for this study. Twenty-four separately dosed water samples were stored for seven days under the conditions listed in the chart that follows:

Storage conditions			Number of
pH	Temperature	Cl_2 (ppm)	samples
2	4°C	0	2
2	4°C	2	2
2	24°C	0	2
2	24°C	2	2
7	4°C	0	2
7	4°C	2	2
7	24°C	0	2
7	24°C	0	2
7	24°C	2	2
10	4°C	0	2
10	4°C	2	2
10	24°C	0	2
10	24°C	2	2

Samples were prepared in one-quart glass bottles by pouring pH-adjusted water into the bottle until about two-thirds full. The sample was then dosed with 100 µL of dosing solution and swirled to mix. The bottle was then either filled with appropriate pH water, leaving small air space, or a predetermined amount of sodium hypochlorite was added before the bottle was filled with water. The bottles were capped with aluminum-lined caps and care was taken that water samples did not contact the aluminum liner for the duration of the test. The bottles were wrapped in aluminum foil to protect from light and were either kept in the laboratory at 24°C for seven days or were placed in the cold room at 4°C for seven days, according to the storage plan.

After seven days, the contents of the bottle were transferred to a one-liter Erlenmeyer and made to one liter by addition of a small amount of appropriate pH-adjusted water (pH 2, 7, or 10). The one-liter water sample was then transferred to a two-liter separatory funnel. A 60-mL volume of DCM was used to rinse the bottle and the Erlenmeyer and finally transferred to the separatory funnel. The extraction and sample preparation protocol established by the extraction study was used to prepare the sample for GC analysis. The samples were all extracted at their existing pH without the addition of salt. Although the extraction recovery for NDMA is greater when salt is added to the water sample, the overall advantage of salting

the water sample was not considered to be sufficiently great to warrant this additional step.

All samples were analyzed by both TEA and AFID. Of particular interest is the demonstrated loss of NDPhA in the chlorinated sample by the TEA. The recovery data for the preservation study are presented in Table 6 for TEA and in Table 7 for AFID.

Data Analysis-Preservation--

The results of the preservation study were examined on two bases. First, the percent recoveries were submitted to an ANOVA procedure to determine which of the factors affected the recovery upon storage. Second, the recovery relative to the extraction study results was determined to indicate the potential loss upon storage.

The ANOVA for these data is based on a 3 x 2 x 2 factorial experiment with two replicates of each combination. The model is represented by

$$Y_{ijkl} = \mu + \rho_i + \tau_j + \gamma_k + \rho\tau_{ij} + \rho\gamma_{ik} + \tau\gamma_{jk} + \rho\tau\gamma_{ijk} + \varepsilon_{ijkl}$$

where Y_{ijkl} = observed recovery for pH i, temperature j, chlorine level k and repetition l

μ = overall mean

ρ_i = effect of pH i, i = 1, 2, 3

τ_j = effect of temperature j, j = 1, 2

γ_k = effect of chlorine level k, k = 1, 2

$\rho\tau_{ij}$, $\rho\gamma_{ik}$, $\tau\gamma_{jk}$ = two factor interactions

$\rho\tau\gamma_{ijk}$ = three factor interaction

and ε_{ijkl} = random error associated with Y_{ijkl}

All terms, pH, temperature, and chlorine, are considered fixed effects, and all F-ratios calculated against the error mean square. Each compound is investigated separately and the results synthesized to obtain a recommended set of conditions for storage. Tables of the ANOVA's conducted are presented in Appendix D.

NDMA--The results for NDMA on the TEA analyzer were used to generate the ANOVA table. The only significant term in the analysis is pH, but the difference is of a very small order. The increase in recovery obtained by adjusting the pH would be negligible and is not considered of any practical significance.

The results from the AFID showed significant terms of chlorine, as well as pH-chlorine and temperature-chlorine interactions. The absence of

TABLE 6. PRESERVATION STUDY RECOVERY (%) TEA

	NDMA		NDPrA		NDPhA	
	0 ppm Cl_2	2 ppm Cl_2	0 ppm Cl_2	2 ppm Cl_2	0 ppm Cl_2	2 ppm Cl_2
pH 2						
1-24°C	40	40	96	99	31	5
2-24°C	39	40	101	99	31	5
1-4°C	41	44	101	97	49	5
2-4°C	40	40	98	100	50	6
pH 7						
1-24°C	37	36	95	99	79	21
2-24°C	39	41	99	99	59	23
1-4°C	41	38	101	97	86	68
2-4°C	40	38	98	95	87	70
pH 10						
1-24°C	41	41	97	99	65	39
2-24°C	41	41	100	96	74	37
1-4°C	42	41	98	98	87	76
2-4°C	43	41	101	95	88	72

TABLE 7. PRESERVATION STUDY RECOVERY (%) AFID

	NDMA		NDPrA	
	0 ppm Cl_2	2 ppm Cl_2	0 ppm Cl_2	2 ppm Cl_2
pH 2				
1-24°C	37	40	94	93
2-24°C	39	40	100	94
1-4°C	35	40	100	100
2-4°C	38	40	100	94
pH 7				
1-24°C	37	36	89	90
2-24°C	39	40	94	94
1-4°C	41	35	100	96
2-4°C	38	33	100	98
pH 10				
1-24°C	41	39	95	95
2-24°C	40	36	98	92
1-4°C	40	34	98	97
2-4°C	43	34	107	90

chlorine produced a significantly higher recovery, but only 1.8 percent better on average. This is controllable by the addition of sodium thiosulfate, but the overall effect would be small if residual chlorine were present.

The means of the combinations are presented in the table as well, along with their $LSD_{0.05}$'s. As in the chlorine effect, these do not evidence a difference of large enough magnitude to indicate an advantage to any combination of conditions. The $LSD_{0.05}$ for the pH-chlorine combination is 2.5 percent recovery, indicating the best conditions were at pH 7-no chlorine, pH 10-no chlorine and pH 2-2 ppm chlorine, which are indistinguishable from each other. For temperature-chlorine combinations, the $LSD_{0.05}$ was 2.1 percent recovery, which indicated that the 4°C-pH 2 combination was slightly worse than the others. However, only 2-3 percent difference was observed on average.

NDPrA--For NDPrA with the TEA, the ANOVA results (Table A-10) yielded no significant differences among the various storage factors or combinations. For the AFID analyses, the summary of results indicates both temperature and chlorine effects were significant for these analyses. The samples stored at 4°C yielded higher recoveries, 98.3 percent versus 94.0 percent for the 24°C samples. Similarly, the recoveries at 0 ppm chlorine averaged 97.9 versus 94.4 for the 2 ppm data. Although this can be significant, the overall effect is quite low, with a minimum of 94 percent recovery for the worst case.

NDPhA--The analysis of the NDPhA results provided the most information concerning storage conditions for the water samples. In the ANOVA, all factors and interactions were significant, indicating a need for extensive investigation. Since the three-factor interaction was significant, the means for this breakdown were used to perform the evaluation. The $LSD_{0.05}$ for these means is 7.1, and from the values shown in the table, this implies that the best recoveries were pH 7-4°C-0 ppm chlorine and pH 10-4°C-0 ppm chlorine with 86.5 and 87.5, respectively. The conclusion, then, is that storage under nonacidic conditions at cold temperature does best to preserve the nitrosamine content. The recoveries under these same conditions but with 2 ppm residual chlorine in the sample were 69 and 74 percent.

Relative Recoveries--

To determine the effect of storage on the observed results, the average recoveries from the preservation studies were ratioed against the average recoveries from the extraction studies. These results are summarized in Table 8. As can be seen, essentially the same recovery was observed in the preservation studies as in the extraction studies, with NDMA values on the AFID the only ones below 99 percent relative recovery. For these, the data on the TEA make it appear that the detector, not the samples, was the source of the disparity.

TABLE 8. RELATIVE RECOVERY FROM PRESERVATION STUDY

Compound	Detector	Means		Relative recovery (Pres/Ext x 100%)
		Extraction	Preservation	
NDMA	TEA	40.7*	40.2	99
	AFID	41.4*	38.1	92
NDPrA	TEA	98.8	98.3	99
	AFID	97.2	96.2	99
NDPhA	TEA	85.0*	87.0	102

* Without salt.
Best conditions.

PHASE I CONCLUSIONS

Based on the work performed in Phase I of this program, the following conclusions can be made.

1. Nitrosodiphenylamine cannot be gas chromatographed directly. Depending on other factors, its concentration in water can be estimated by gas chromatographic measurement of diphenylamine, using AFID, or direct measurement of NO, using TEA. Both of these compounds are instantaneously formed as a result of thermal degradation of the nitrosodiphenylamine as it is injected onto the gas chromatographic column.

2. Although some problems were encountered with gas chromatography of nitrosodimethylamine using the AFID, the overall results indicate that any of the column packings examined could be used for analysis of NDMA, NDPrA, and diphenylamine. The column packing consisting of 10 percent Carbowax 20M + 2 percent KOH appears to be the best of those tested based primarily on better separation of NDMA from the solvent peak when using the AFID and good peak shape indicating little or no tailing.

3. Extraction of NDMA from unsalted water with DCM is low, approximately 40 percent, but reproducible. The addition of 300 g/L of NaCl improves the extraction efficiency for the NDMA to approximately 68 percent. The extraction of NDPrA from salted or unsalted water with DCM is essentially 100 percent. The extraction efficiency for NDPhA from salted or unsalted water with DCM appears to be about 80-85 percent; solvent evaporation data indicate there may be some degradation of NDPhA during evaporation which may account for the bulk of the 15 percent loss.

4. Preservation studies indicate essentially no loss of NDMA and NDPrA when stored for seven days at a pH of 2, 7, or 10 at 24°C or 4°C with 0 or 2 ppm free chlorine but stored protected from light. The best water storage conditions for NDPhA and therefore for water samples for nitrosamine analysis, are at a pH of 7 to 10, at 4°C, protected from light, and with removal of any residual chlorine.

SECTION 5

PHASE II

OBJECTIVES

The objectives of Phase II were as follows:

1. Investigation of two liquid-solid chromatographic cleanup procedures, including the determination of the separation patterns and recoveries for all of the substances of interest.
2. Application of the method to five wastewater samples from industrial discharges. This aspect of the work included modifications of the method as required to analyze the wastewaters. These would be based on experience gained earlier in the program and on new developments reported in scientific literature.
3. Generation of accuracy and precision data for all substances of interest by dosing them into the five wastewaters. Determinations would be made immediately after dosing and after one week of storage at the best conditions observed in Phase I.

PROGRAM DIRECTION

Representatives of SwRI met with the project officer in Cincinnati before Phase II work commenced. Discussion with respect to Phase II was primarily on the cleanup columns and procedures to be used. Florisil and alumina were selected as the adsorptive solids. Fractionation of compounds was limited to that which can be obtained on the above-named solids. Fractionation on secondary columns such as silica gel or charcoal was considered to be beyond the scope of this program.

EXPERIMENTATION

During the Phase I efforts, it was determined that NDPhA was decomposed in the injector and that DPhA subsequently eluted from the column and was detected by the AFID. Injected DPhA eluted at the same time and therefore it was not possible to identify NDPhA in the presence of DPhA. The liquid-solid column chromatography cleanup effort, in addition to providing a general cleanup procedure for the nitrosamines of interest, must also specifically provide for separation of NDPhA and DPhA. Only when these two compounds are collected in different fractions can they be determined by gas chromatography with the AFID.

The TEA determination of NDPhA by detection of the NO liberated in the injector, as was performed in Phase I, is only satisfactory with clean samples and standards.

Since it was anticipated that the AFID would be the desired detector for monitoring purposes, the column cleanup efforts were addressed specifically to the use of this detector. The TEA requires essentially no cleanup and can be used with most common solvents.

Liquid-Solid Column Chromatography

The literature search revealed no information relative to cleanup and separation procedures and some preliminary investigations with both Florisil and alumina were required.

Cleanup procedures for both Florisil and alumina were developed which satisfied the basic requirements of recovery of NDMA and NDPrA and enabled recovery of NDPhA separated from DPhA. The detailed Florisil and alumina cleanup procedures for the nitrosamines are presented in Appendix E.

Florisil--

The Florisil cleanup procedure results in the collection of NDMA, NDPrA, and NDPhA in Fraction II, a single fraction free of DPhA. The DPhA can be partially recovered in Fraction I if desired.

Five doses of each of the three nitrosamines and two doses of the DPhA were applied to the Florisil column and recovered. The dosing levels and recoveries from the Florisil cleanup procedure are presented in Table 9. Although the recovery of DPhA in Fraction I is low, it was confirmed in the preliminary work that no DPhA was carried over into Fraction II. The recoveries of NDMA, NDPrA, and NDPhA appear to be excellent and consistent with recoveries from the Kuderna-Danish concentration data.

TABLE 9. RECOVERY (%) FROM FLORISIL COLUMN

	Fraction I				Fraction II			
Sample	NDMA	NDPrA	NDPhA	DPhA	NDMA	NDPrA	NDPhA	DPhA
1	0	0	--	0	90	104	92	--
2	0	0	--	3	98	100	87	--
3	0	0	--	0	101	99	92	--
4	0	0	--	44	102	100	69	--
5	0	0	--	33	101	103	87	--
Avg.	0	0	--	39	98	101	85	--

Amount Added: NDMA 0.800 µg NDPhA 16.0 µg
NDPrA 2.40 µg DPhA 16.0 µg (Samples 4 and 5 only

Alumina--

The alumina cleanup procedure requires that two fractions be collected, since the separation pattern is different from the Florisil cleanup procedure. With the alumina cleanup procedure, Fraction I contains all of the NDPhA and a portion of the NDPrA. Fraction II contains all of the NDMA, most of the NDPrA, and the DPhA.

Five doses of each of the three nitrosamines and two doses of the DPhA were applied to the alumina column and recovered. The dosing levels and recoveries from the alumina cleanup procedure are presented in Table 10. Recoveries of all three nitrosamines from the alumina cleanup procedure were very good although the NDPrA may appear in both fractions.

TABLE 10. RECOVERY (%) FROM ALUMINA COLUMN

	Fraction I				Fraction II			
Sample	NDMA	NDPrA	NDPhA	DPhA	NDMA	NDPrA	NDPhA	DPhA
1	0	0	103	--	102	100	--	8
2	0	0	98	--	98	100	--	0
3	0	0	98	--	96	94	--	3
4	0	6	95	--	98	88	--	94
5	0	8	95	--	92	88	--	102
Avg.	0	-	98	--	97	98*	--	97

Amount Added: NDMA 0.400 µg NDPhA 9.0 µg
NDPrA 1.20 µg DPhA 9.0 µg (Samples 4 and 5 only)

* Includes NDPrA from Fraction I.

Wastewater Application

With the assistance and approval of the project officer, five wastewaters were procured and analyzed. All samples were put in clean one-gallon bottles and shipped, unrefrigerated, via air to Southwest Research Institute laboratories. Upon receipt at SwRI, the samples were pooled and adjusted to pH 7-9 if necessary, returned to the bottles in which they were shipped and stored at 4°C in the dark until used.

Analysis of Wastewaters--

Analysis for NDPhA via DPhA in wastewaters cannot be accomplished with the TEA and therefore the NDPhA was determined by AFID only. NDMA and NDPrA can be determined by both TEA and AFID. In addition, NDMA and NDPrA can be determined quite selectively by the Hall Electrolytic Conductivity Detector when used in the pyrolytic mode (Reference 50). The TEA and the Hall (pyrolytic mode) are not appreciably affected by the solvent and do not require a solvent transfer.

The clean water extraction procedure was modified slightly by inclusion of an aqueous-acid wash of the DCM to remove amines. The "Extraction and Cleanup Procedure for Wastewaters" as employed on the five wastewaters is presented in Appendix E.

The protocol for wastewater sample analysis was as follows:

1. Extract sample with 3 x 60 mL volumes of DCM and acid wash.
2. Concentrate DCM to 10 mL.
3. Analyze for NDMA and NDPrA with both TEA and Hall (no cleanup).
4. Concentrate DCM to 2 mL.
5. Clean up sample using Florisil or alumina.
6. Analyze appropriate fraction(s) for NDPhA using AFID.
7. Analyze appropriate fraction(s) for NDMA and NDPrA using AFID.

In order to develop method improvements and to provide base data for the dosing and recovery experiments and the accuracy and precision evaluations to follow, each wastewater was analyzed in triplicate for each compound of interest in this program.

Each wastewater was subjected to a preliminary examination to determine which cleanup method, Florisil or alumina, should be used. With all wastewaters, the alumina was found to be marginally more effective and was used for all sample cleanup requirements. It should be noted that most of the TEA and Hall analyses for NDMA and NDPrA were performed on samples which were not cleaned up and it was necessary to perform only one analysis. An AFID analysis was required of each of the two fractions since the NDPrA was usually in both fractions. It was necessary to perform another analysis with the AFID for the NDPhA determination. For data tabulation, the NDPrA reported represents the sum of the NDPrA in Fraction I and Fraction II. The results of the analysis of the five wastewaters are presented in Tables 11 through 15. Appendix F shows chromatograms obtained of the wastewater extracts with the different detectors.

No particularly difficult problems were encountered with any of the wastewater samples and the results obtained with the different detectors was quite consistent and in good agreement with each other. Wastewaters 1, 3 and 5 all showed small amounts of NDMA ranging from 0.1 to 0.4 µg/L by all three detection systems. Wastewater 1 showed small amounts of NDPrA, 0.5 µg/L by all three detector systems whereas Wastewater 3 showed 0.2 µg/L for NDPrA by Hall only. Wastewater 2 showed a trace, 0.4 µg/L, of NDPhA.

Although the tabulated data indicate any of the detectors could be used satisfactorily, examination of the chromatograms indicates the AFID is more prone to interferences and possible misinterpretation than either of the other detectors.

TABLE 11. ANALYSIS OF WASTEWATER 1

Replicate	NDMA (µg/L) AFID	NDMA (µg/L) TEA*	NDMA (µg/L) Hall*	NDPrA (µg/L) AFID	NDPrA (µg/L) TEA*	NDPrA (µg/L) Hall*	NDPhA (µg/L) AFID
1	0.26	0.34	0.32	0.48	0.53	0.56	0
2	0.23	0.34	0.31	0.56	0.49	0.57	0
3	0.23	0.31	0.31	0.44	0.51	0.53	0
Average	0.24	0.33	0.31	0.49	0.51	0.55	0

* No cleanup on samples analyzed by TEA and Hall.

TABLE 12. ANALYSIS OF WASTEWATER 2

Replicate	NDMA (µg/L) AFID	NDMA (µg/L) TEA*	NDMA (µg/L) Hall*	NDPrA (µg/L) AFID	NDPrA (µg/L) TEA*	NDPrA (µg/L) Hall*	NDPhA (µg/L) AFID
1	0	0	0	0	0	0	0.33
2	0	0	0	0	0	0	0.43
3	0	0	0	0	0	0	0.54
Average	0	0	0	0	0	0	0.43

* No cleanup on samples analyzed by TEA and Hall.

TABLE 13. ANALYSIS OF WASTEWATER 3

Replicate	NDMA (µg/L) AFID	NDMA (µg/L) TEA*	NDMA (µg/L) Hall*	NDPrA (µg/L) AFID	NDPrA (µg/L) TEA*	NDPrA (µg/L) Hall*	NDPhA (µg/L) AFID
1	0.29	(0.31)	0.34	0	0	0	0
2	0.29	(0.30)	0.37	0	0	0	0
3	0.27	(0.30)	0.36	0	0	0	0
Average	0.28	(0.30)	0.36	0	0	0	0

* No cleanup on samples analyzed by TEA and Hall.

TABLE 14. ANALYSIS OF WASTEWATER 4

Replicate	NDMA (µg/L)			NDPrA (µg/L)			NDPhA (µg/L)
	AFID	TEA*	Hall*	AFID	TEA*	Hall*	AFID
1	0	0	0	0	0	0.24	0
2	0	0	0	0	0	0.16	0
3	0	0	0	0	0	0.27	0
Average	0	0	0	0	0	0.22	0

* No cleanup on samples analyzed by TEA and Hall.

TABLE 15. ANALYSIS OF WASTEWATER 5

Replicate	NDMA (µg/L)			NDPrA (µg/L)			NDPhA (µg/L)
	AFID	TEA*	Hall*	AFID	TEA*	Hall*	AFID
1	0.21	0.16	0.10	0	0	0	0
2	0.21	0.16	0.14	0	0	0	0
3	0.20	0.15	0.10	0	0	0	0
Average	0.21	0.16	0.11	0	0	0	0

* No cleanup on samples analyzed by TEA and Hall.

Dosing of Wastewaters and Recovery Analyses--

In order to accumulate data for accuracy and precision evaluations, each of six one-liter portions of each wastewater were dosed with 100 µL of an acetone solution containing 0.8 µg NDMA, 1.20 µg NDPrA, and 9.0 µg NDPhA per 100 µL. Three of these dosed samples were analyzed immediately and three were stored in the dark for seven days in sealed glass containers at 4°C.

The results of the zero and seven day recovery experiments are shown in Tables 16 through 22.

Accuracy and Precision

Approach--

The accuracy and precision assessment for the method was of a limited nature due to the number and types of analytical results obtained. According to the design of the program three replicates of each of five wastewaters were to be dosed for the compounds of interest and analyzed, both at a zero time and after seven days' storage under the appropriate conditions. This provided 15 determinations of recovery of the dose for statistical analyses. However, the five wastewaters could not be considered equivalent, neither in their nature nor in the behavior of the method when applied. As a result, combining the results to form an overall evaluation of the method was inappropriate. Instead, the results of each wastewater were treated individually and no general statement about the method was attempted.

The accuracy of a method refers to its ability to obtain on average the true value, within the limits of the variability inherent in the method. For this study, the accuracy standard used was the percent recovery of the amount of the particular nitrosamine dosed into the wastewater, with 100 percent representing an accurate method. Precision relates to the variability among measurements arising from various sources and is considered separately from the accuracy evaluation. It is possible for a method to be precise, meaning having little variability among measurements, without being considered accurate. For this study, the precision measurement used was the range or the difference between the highest and lowest recoveries. While the range is sensitive to single extreme values, it was deemed more appropraite for these data than other measures, given the number of observations available.

The recoveries obtained after seven days' storage were examined separately to evaluate the effects of storage on the observed levels of these compounds. These recoveries are based on the total amount present at time zero rather than the dosing level, since it can be assumed that any degradation would affect both the initial amount and the dosed amount in equal proportions.

Where the analyses were run on more than one detector, these results were treated individually and compared for their consistency. There were differences noted in the results obtained from one detector to another,

TABLE 16. RECOVERY OF NITROSODIMETHYLAMINE FROM DOSED WASTEWATERS (AFID Detection)

Time (days)	Replicate	Wastewater 1	2	3	4	5
		% Recovery of dosed amount				
0	1	32	38	31	29	30
	2	32	38	34	30	24
	3	32	--	36	31	22
	Avg.	32	38	34	30	25
	Range	0	0	5	2	8
		% Recovery of 7-day preservation sample relative to 0-day average				
7	1	84	50	118	137	127
	2	90	74	115	143	159
	3	84	58	113	110	105
	Avg.	86	61	115	130	132
	Range	6	24	5	33	54

TABLE 17. RECOVERY OF NITROSODIMETHYLAMINE FROM DOSED WASTEWATERS (TEA Detection)

Time (days)	Replicate	Wastewater 1	2	3	4	5
		% Recovery of dosed amount				
0	1	42	46	23*	48	41
	2	40	57	28*	49	39
	3	38	--	53*	45	35
	Avg.	40	52	35*	47	38
	Range	4	--	30*	4	6
		% Recovery of 7-day preservation sample relative to 0-day average				
7	1	--**	68	138	102	111
	2	--	84	134	96	111
	3	--	54	131	96	109
	Avg.	--	68	134	98	111
	Range	--	30	7	6	2

* After cleanup.
** Instrument difficulties.

TABLE 18. RECOVERY OF NITROSODIMETHYLAMINE FROM DOSED WASTEWATERS
(Hall Detection)

Time (days)	Replicate	Wastewater 1	2	3	4	5
		% Recovery of dosed amount				
0	1	40	45	50	34	39
	2	46	48	48	34	43
	3	40	48	51	36	42
	Avg.	42	47	50	35	41
	Range	6	3	3	2	4
		% Recovery of 7-day preservation sample relative to 0-day average				
7	1	92	--*	103	97	100
	2	92	--	112	103	95
	3	89	--	109	100	95
	Avg.	91	--	108	100	98
	Range	3	--	9	6	5

* Not calculable due to interference.

TABLE 19. RECOVERY OF NITROSODIPROPYLAMINE FROM DOSED WASTEWATERS (AFID Detection)

Time (days)	Replicate	Wastewater 1	2	3	4	5
		% Recovery of dosed amount				
0	1	81	52	83	107	77
	2	76	55	79	117	81
	3	85	--	84	86	81
	Avg.	81	54	82	103	80
	Range	9	3	5	31	4
		% Recovery of 7-day preservation sample relative to 0-day average				
7	1	97	46	115	95	103
	2	102	80	120	76	114
	3	88	57	113	83	108
	Avg.	96	61	116	84	108
	Range	14	34	7	19	11

TABLE 20. RECOVERY OF NITROSODIPROPYLAMINE FROM DOSED WASTEWATERS (TEA Detection)

Time (days)	Replicate	Wastewater 1	2	3	4	5
		% Recovery of dosed amount				
0	1	93	71	100	104	99
	2	100	84	102	108	96
	3	93	--	102	99	99
	Avg.	95	78	101	104	98
	Range	7	13	2	9	3
		% Recovery of 7-day preservation sample relative to 0-day average				
7	1	--*	53	97	98	102
	2	--	65	104	100	105
	3	--	90	107	100	102
	Avg.	--	69	103	99	103
	Range	--	37	10	2	3

* Instrument difficulties.

TABLE 21. RECOVERY OF NITROSODIPROPYLAMINE FROM DOSED WASTEWATERS (Hall Detection)

Time (days)	Replicate	Wastewater 1	2	3	4	5
		% Recovery of dosed amount				
0	1	94	74	106	105	93
	2	98	70	104	113	91
	3	90	74	106	107	103
	Avg.	94	73	105	108	96
	Range	8	4	2	8	12
		% Recovery of 7-day preservation sample relative to 0-day average				
7	1	96	--*	100	112	96
	2	96	--	102	114	115
	3	89	--	98	112	113
	Avg.	93	--	100	113	107
	Range	7	--	4	2	19

* Not calculated due to interference.

TABLE 22. RECOVERY OF NITROSODIPHENYLAMINE FROM DOSED WASTEWATERS (AFID Detection)

Time (days)	Replicate	Wastewater 1	2	3	4	5
		% Recovery of dosed amount				
0	1	51	8	50	80	89
	2	35	5	50	92	90
	3	51	--	55	92	88
	Avg.	46	6	52	88	89
	Range	16	3	5	12	2
		% Recovery of 7-day preservation sample relative to 0-day average				
7	1	89	167	171	98	98
	2	87	167	177	98	93
	3	87	130	169	99	99
	Avg.	87	154	173	98	97
	Range	2	37	8	1	6

partially due to the sample preparation step involved. For the TEA and Hall detectors, no cleanup of the sample was necessary, but cleanup was required for analysis by AFID. As a result, there was some apparent loss of material, and the results for the AFID detector generally ran 70 to 80 percent of those from the other detectors.

Results--

Wastewater 1--The analyses run on Wastewater 1 were generally good for NDMA and NDPrA, but low on NDPhA. The average recoveries of 40 and 42 percent for NDMA on the TEA and Hall, respectively, are equivalent to the recoveries from clean water in Phase I. The 32 percent recovery obtained using the AFID likely represents loss on cleanup, as discussed above, as opposed to less efficient detection. For NDPrA, similarly, recoveries of 94 and 95 percent on the TEA and Hall and 81 percent for the AFID are equivalent to the Phase I results when the effect of the cleanup step is considered for the AFID. There was not good recovery for NDPhA, with only 46 percent on average, much lower than would be expected from the Phase I study if no problems existed. The preservation recoveries were good, ranging from 86 percent for NDMA on the AFID to 96 percent for NDPrA on the AFID. It is indicated that storing this wastewater for analysis for nitrosamines would not result in a serious loss of material.

The precision of these determinations was reasonable, with most triplicate analyses having a range of 6 to 9 percent recovery, with some ranges both above and below that figure. The ranges for the preservation recoveries were similar to those for the initial analyses and can be considered indicative of method variability alone. The median range was 7 percent recovery for the 12 sets of determinations.

Wastewater 2--The results for the analyses on Wastewater 2 were inconsistent for the three nitrosamines under study. Analyses for NDMA gave higher recoveries than were obtained on Wastewater 1 and higher than expected based on the Phase I results. For the NDPrA, on the other hand, only about 75 percent was recovered on the TEA and Hall detectors, for the samples prior to cleanup, and less on the cleaned up sample processed through the AFID. The recovery of NDPhA from this wastewater was poor, with an average recovery barely distinguishable from zero.

The preservation recoveries were unsatisfactory for all three nitrosamines, with average recoveries of 60 to 70 percent for NDMA and NDPrA and considerable variability. The 154 percent for NDPhA results from the low level observed initially and is not considered indicative. The conclusion is that storage of this type of wastewater for nitrosamine analysis will produce unreliable results.

The precision of the method can be evaluated using only the initial analyses for this wastewater. The variability was acceptable, with the range of the triplicate determinations going from 0 for NDMA-AFID to 13 for NDPrA-TEA. The median range was 3 percent recovery, which appeared in three of the seven sets of data.

Wastewater 3--The analyses for the nitrosamines of interest in Wastewater 3 provided good results in the initial period for both NDMA and NDPrA, with the exception of the NDMA-TEA results. These were the only runs made on the TEA with samples after cleanup, and there was apparently a problem with the analyses and these were considered nontypical. The results of the NDMA analyses on the AFID and Hall detectors indicate there was no problem in extracting and recovering the substances of interest in this wastewater. The recovery of NDPrA in this wastewater was complete for the sample prior to cleanup and as high as can be expected for the sample after cleanup using the AFID. The NDPhA recovery, consistent with the previous wastewaters, was low, with only 52 percent recovered on average.

The preservation recoveries were at or above 100 percent in all cases, with the highest value for NDPhA of 173 percent. This represents 90 percent of the original spike level, however, and may indicate a problem with the initial analyses.

The precision of the initial analyses was very good, with a range of 5 percent recovery including all triplicate analyses except the NDMA-TEA. The precision of the preserved samples was acceptable, but was greater than for the initial analyses. Typical ranges for these triplicate determinations were from 7 to 9 percent recovery and may indicate greater variability among measurements resulting from storage of the samples.

Wastewater 4--The analytical results from this wastewater were generally good for all three nitrosamines studied. The average recoveries for NDMA on both TEA and Hall were close to the expected maximum based on the Phase I results and for the AFID, the recovery was 73 percent of their average, which is a typical result after sample cleanup. The NDPrA results were good on both the TEA and Hall, but higher than expected on the AFID after consideration of loss on cleanup. The recovery of NDPhA was good for this wastewater, with 88 percent recovery of the initial dosed amount. In general, the method performed with reasonable accuracy in this wastewater. The preservation results were not as consistent, with 100 to 130 percent recovery for NDMA and 84 to 113 percent recovery for NDPrA among the detectors used. Recovery of NDPhA was nearly 100 percent for the preserved samples.

There was considerable scatter among the precision components in this wastewater as well. The ranges among the initial analyses were below 10 percent recovery with the exception of the NDPrA-AFID, with a range of 31, and the NDPhA with a range of 12 percent recovery. The NDPrA-AFID result coupled with the higher than expected recovery indicates that there was some problem in those analyses and that those results should not be considered representative of the wastewater. Ranges among the preservation recoveries were similar, with values as low as one and as high as 33, but for the majority of the sets of triplicates, the repeatability was good.

Wastewater 5--The analyses of Wastewater 5 at time zero were the most consistent of the data generated. Both TEA and Hall gave average recoveries around 40 percent for NDMA and 100 percent for NDPrA, consistent

with the Phase I results. The AFID results were lower due to the cleanup step but were in line with what would be expected. The NDPhA average recovery was 89 percent at the initial analyses. Preservation recoveries were generally higher than the initial analysis and less consistent.

The precision of the initial analyses was good, with ranges from 2 to 12 percent recovery and a median range of 4 percent recovery. As in the previous data sets, less precise results could be expected from the preserved samples which indicates additional variability arising from the wastewater itself and not due to the method. Thus from the standpoint of precision as well as accuracy, storage of samples for nitrosamine analysis cannot be recommended.

SUMMARY OF PHASE II

Fully activated Florisil PR and alumina (Basic) with 2 percent water were evaluated as column chromatography cleanup materials and as a means of separating NDPhA from DPhA. Both materials were found to be satisfactory in recovery of all three nitrosamines in separation of NDPhA from DPhA. With wastewater extracts, the alumina proved to be somewhat more efficient in removal of early eluting interferences. The alumina cleanup column was used to clean up all wastewater extracts.

Five wastewater samples were analyzed. NDMA and NDPrA were determined by direct injection of the DCM-concentrated extract requiring no cleanup. NDMA, NDPrA and NDPhA were determined by AFID on the extracted samples after alumina column cleanup and fractionation. Recovery data on the dosed wastewater immediately after dosing, and after seven days' storage at 4°C were determined.

Results of the analysis of wastewaters indicated AFID analysis for NDMA and NDPrA on cleanup samples generally gave results which were slightly lower than the results obtained with TEA and Hall on extracts having no cleanup. The recoveries of all the nitrosamines from the wastewaters, and especially the NDPhA, vary considerably, showing the effects of the wastewater matrix on the nitrosamines introduced into the wastewater.

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APPENDIX A

GAS CHROMATOGRAPHY

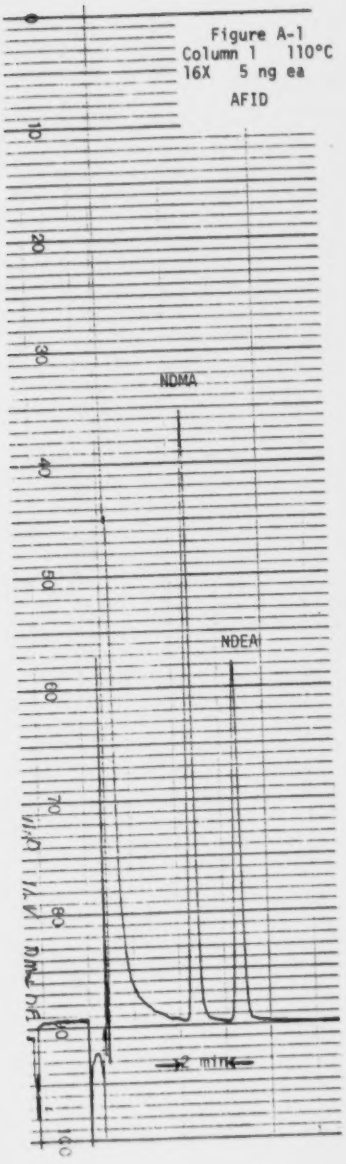


Figure A-1
Column 1 110°C
16X 5 ng ea
AFID
NDMA
NDEA
2 min

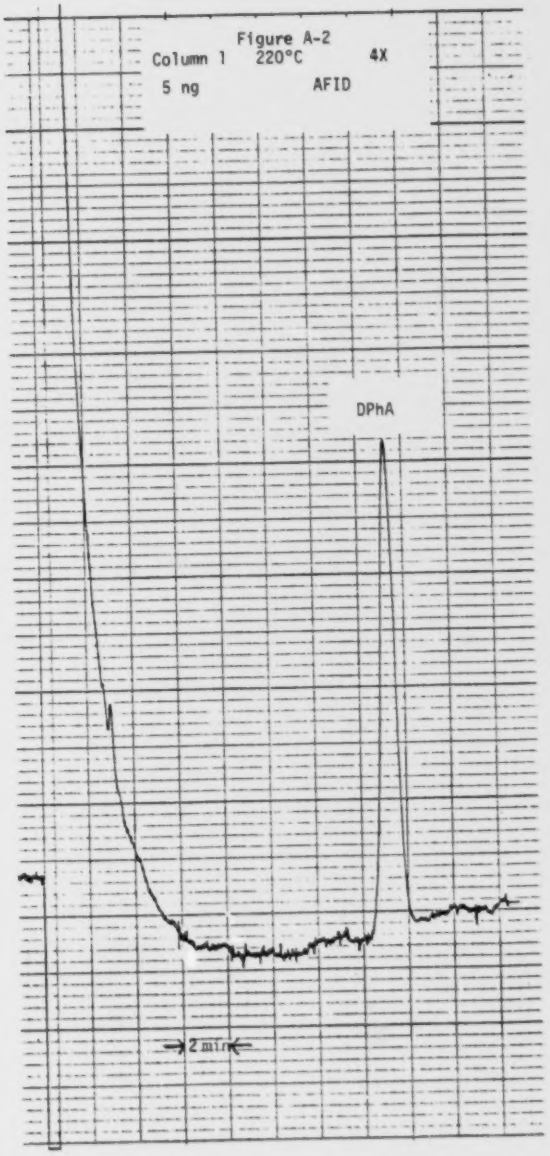


Figure A-2
Column 1 220°C 4X
5 ng AFID
DPhA
2 min

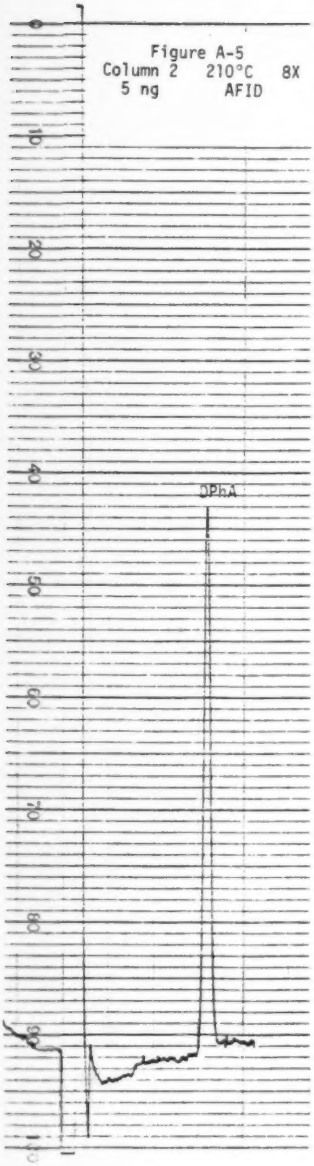


Figure A-5
Column 2 210°C 8X
5 ng AFID
DPhA

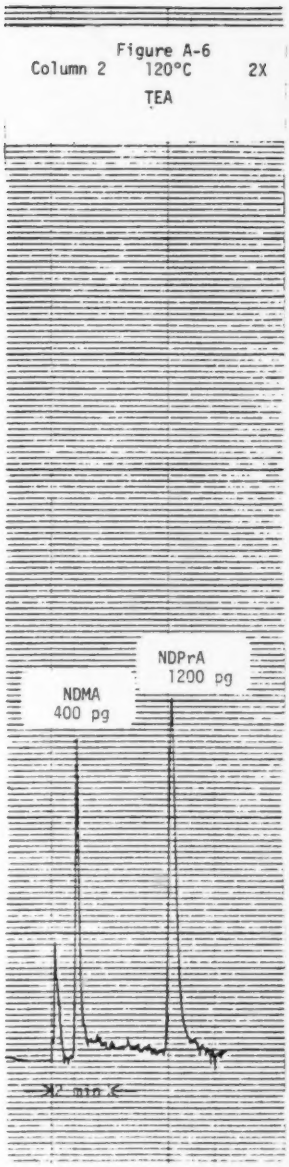


Figure A-6
Column 2 120°C 2X
TEA
NDPrA
1200 pg
NDMA
400 pg
2 min

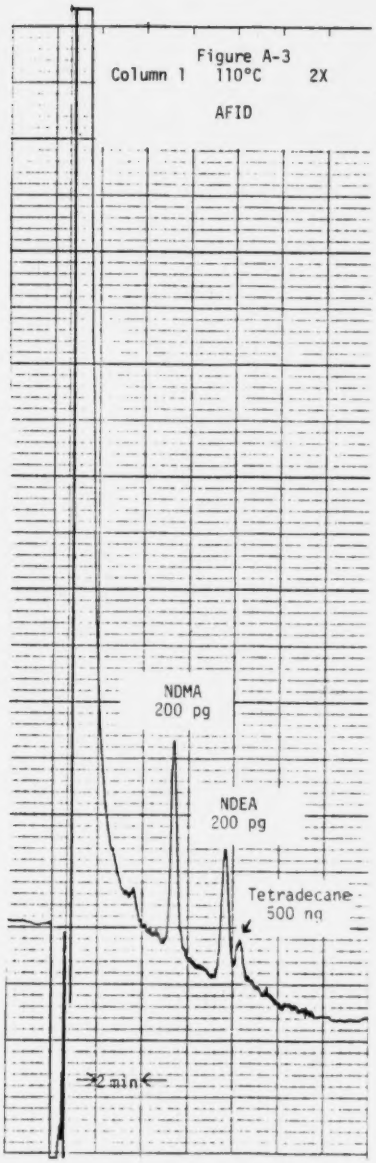


Figure A-3
Column 1 110°C 2X
AFID
NDMA
200 pg
NDEA
200 pg
Tetradecane
500 ng
2 min

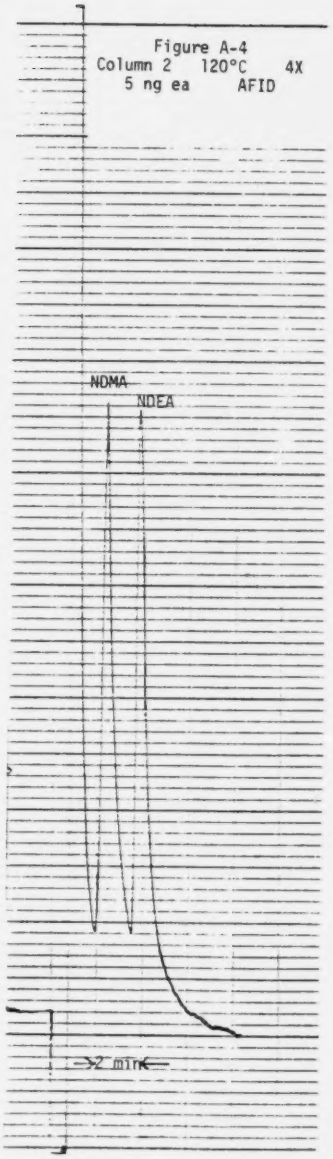


Figure A-4
Column 2 120°C 4X
5 ng ea AFID
NDMA
NDEA
2 min

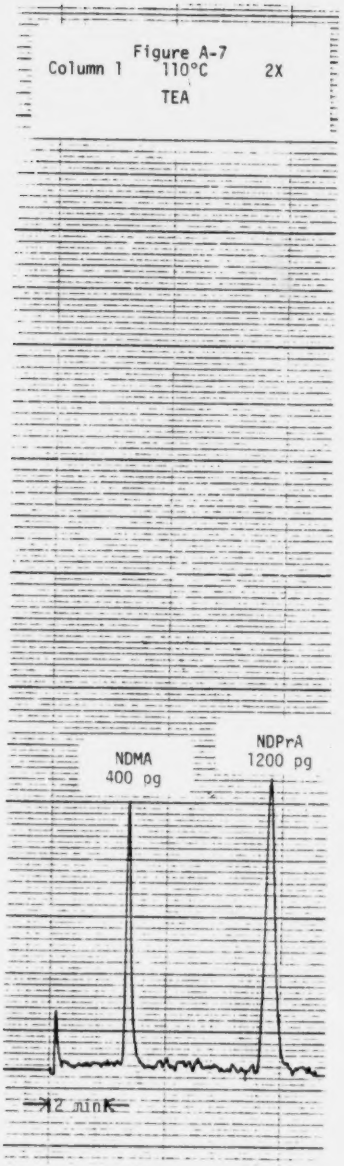


Figure A-7
Column 1 110°C 2X
TEA
NDMA
400 pg
NDPrA
1200 pg
2 min

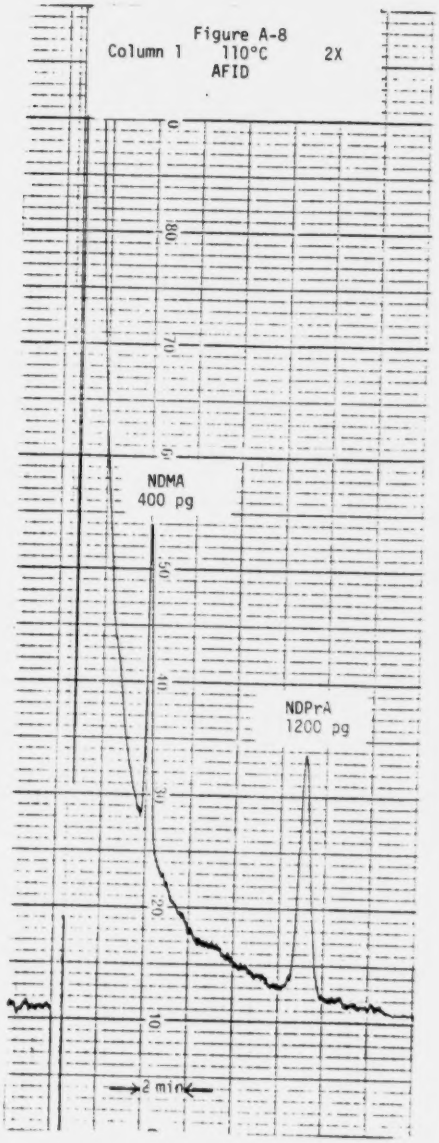


Figure A-8
Column 1 110°C 2X
AFID
NDMA
400 pg
NDPrA
1200 pg
2 min

APPENDIX B

EXTRACTION RECOVERY

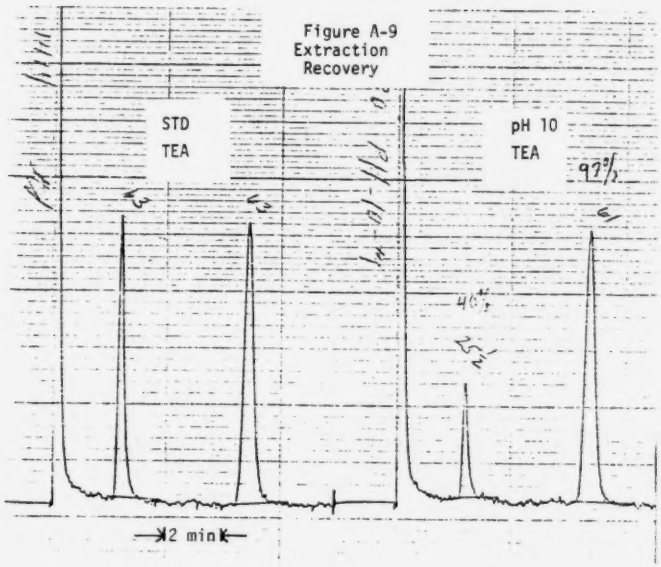
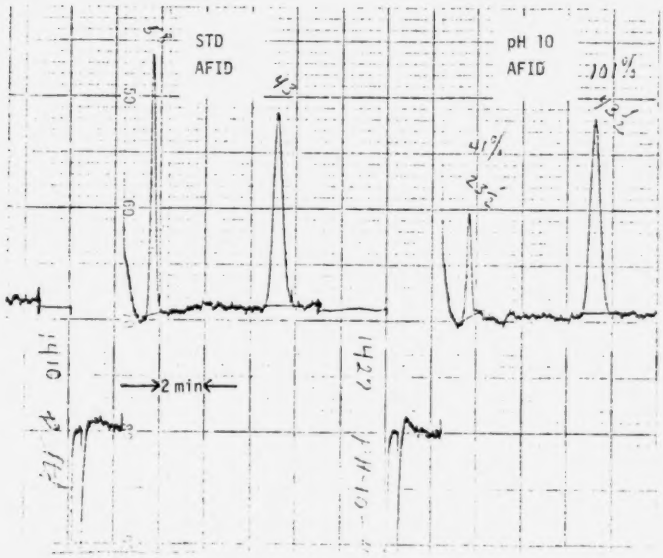


Figure A-9
Extraction
Recovery



APPENDIX C

ANOVA TABLES--EXTRACTION

TABLE A-1. STATISTICAL SUMMARY FOR NDMA ON TEA DETECTOR
Extraction Study

ANOVA TABLE

Source	df	SS	MS	F
Salt	1	3618.1	3618.1	1247.6 *
pH's	2	0.4	0.2	0.1
Interaction	2	1.9	1.0	0.3
Error	14	40.2	2.9	
Total	19	3660.6		

Means

Salt

1 (none)	2 (added)
40.7	67.6

pH

2	7	10
54.3	54.0	54.2

Interaction

(1,2)	(1,7)	(1,10)	(2,2)	(2,7)	(2,10)
41.3	40.3	40.7	67.3	67.8	67.7

* significant at 0.05 level.

TABLE A-2 STATISTICAL SUMMARY FOR NDMA on AFID DETECTOR
Extraction Study

ANOVA TABLE

Source	df	SS	MS	F
Salt	1	4470.1	4470.1	1117.5 *
pH's	2	22.0	11.0	2.8
Interaction	2	58.7	29.4	7.4
Error	14	55.8	4.0	
Total	19	4606.6		

Means

Salt

1 (none)	2 (added)
41.4	71.3

pH

2	7	10
55.2	56.1	57.8

Interaction

(1,2)	(1,7)	(1,10)	(2,2)	(2,7)	(2,10)
42.7	40.8	41.0	67.7	71.5	74.7

* significant at 0.05 level

TABLE A-3. STATISTICAL SUMMARY FOR NDPrA on TEA DETECTOR
Extraction Study

ANOVA TABLE

Source	df	SS	MS	F
Salt	1	5.0	5.0	2.9
pH's	2	4.0	2.0	1.2
Interaction	2	14.7	7.4	4.4
Error	14	23.5	1.7	
Total	19	47.2		

Means

Salt

1 (none)	2 (added)
97.3	98.3

pH

2	7	10
97.2	98.3	97.8

Interaction

(1,2)	(1,7)	(1,10)	(2,2)	(2,7)	(2,10)
97.7	96.8	97.7	96.7	99.8	98.0

TABLE A-4. STATISTICAL SUMMARY FOR NDPrA for AFID DETECTOR
Extraction Study

ANOVA TABLE

Source	df	SS	MS	F
Salt	1	0.5	0.5	0.1
pH's	2	55.0	27.5	6.3 *
Interaction	2	32.0	16.0	3.6
Error	14	61.1	4.4	
Total	19	148.6		

Means

Salt

1 (none)	2 (added)
97.0	97.3

pH

2	7	10
98.3	95.1	98.7

Interaction

(1, 2)	(1,7)	(1,10)	(2,2)	(2,7)	(2, 10)
99.7	93.5	99.0	97.0	96.8	98.3

* significant at 0.05 level

TABLE A-5. STATISTICAL SUMMARY FOR NDPhA on TEA DETECTOR
Extraction Study

ANOVA TABLE

Source	df	SS	MS	F
Salt	1	140.5	140.5	16.9 *
pH's	2	12.0	6.0	0.7
Interaction	2	24.3	12.2	1.5
Error	14	115.8	8.3	
Total	10	292.6		

Means

Salt

1 (none)	2 (added)
85.0	79.7

pH

2	7	10
81.3	82.4	83.3

Interaction

(1,2)	(1,7)	(1,10)	(2,2)	(2,7)	(2,10)
83.3	84.3	87.7	79.3	80.5	79.0

* significant at 0.05 level

APPENDIX D

ANOVA TABLES--PRESERVATION

TABLE A-6. STATISTICAL SUMMARY FOR NDMA ON TEA ANALYZER
Preservation Study

ANOVA Table

Source	df	SS	MS	F
pH	2	28.58	14.29	7.00 *
Temperature	1	7.04	7.04	3.45
Chlorine	1	0.38	0.38	< 1
pH x T	2	0.58	0.29	< 1
pH x C	2	4.75	2.38	1.16
T x C	1	2.04	2.04	1.00
pH x TXC	2	4.08	2.04	1.00
Residual	12	24.50	2.04	
Total	23			

Mean = 40.2

Cell Means

pH (LSD = 1.6)

2	7	10
40.5	38.8	41.4

TABLE A-7. STATISTICAL SUMMARY FOR NDMA ON AFID ANALYZER
Preservation Study

ANOVA TABLE

Source	df	SS	MS	F
pH	2	7.00	3.50	1.26
Temperature	1	7.04	7.04	2.53
Chlorine	1	18.38	18.38	6.61*
pH x T	2	0.33	0.17	<1
pH x C	2	67.00	33.50	12.05*
T x C	1	12.04	12.04	4.33*
pH x TXC	2	14.33	7.17	2.58
Residual	12	32.55	2.71	
Total	23			

Mean = 38.1

Cells

Chlorine

0	2
39.0	37.2

pH-Chlorine (LSD = 25)

(2,0)	(2,2)	(7,0)	(7,2)	(10,0)	(10,2)
37.2	40.0	38.8	36.0	41.0	35.8

Temp-Chlorine (LSD = 2.1)

(4,0)	(24,0)	(4,2)	(24,2)
39.2	38.8	36.0	38.5

* significance at 0.05 level

TABLE A-8. STATISTICAL SUMMARY FOR NDPrA ON AFID ANALYZER
Preservation Study

ANOVA TABLE

Source	df	SS	MS	F
pH	2	13.58	6.79	<1
Temperature	1	112.67	112.67	10.17*
Chlorine	1	73.50	73.50	6.63*
pH x T	2	17.58	8.79	<1
pH x C	2	22.75	11.38	1.03
T x C	1	13.50	13.50	1.22
pH x TXC	2	10.75	5.38	<1
Residual	12	133.00	11.08	
Total	23			

Mean = 96.2

Cells

Temp.

4°	24°
98.3	94.0

Chlorine

0	2
97.9	94.4

* significant at 0.05 level

TABLE A-9. STATISTICAL SUMMARY FOR NDPrA ON TEA ANALYZER
Preservation Study

ANOVA TABLE

Source	df	SS	MS	F
pH	2	4.75	2.38	<1
Temperature	1	0.00	0.00	<1
Chlorine	1	6.00	6.00	1.33
pH x T	2	0.25	0.13	<1
pH x C	2	3.25	1.63	<1
T x C	1	13.50	13.50	3.00
pH x TXC	2	4.75	2.38	<1
Residual	12	54.00	4.50	
Total	23			

Mean = 98.3

TABLE A-10. STATISTICAL SUMMARY FOR NDPhA ON TEA ANALYZER
Preservation Study

ANOVA TABLE

Source	df	SS	MS	F
pH	2	9395.08	4697.54	220 *
Temperature	1	3151.04	3151.04	147 *
Chlorine	1	5370.04	5370.04	251 *
pH x T	2	567.58	283.79	13.28*
pH x C	2	172.58	86.29	4.40*
T x C	1	145.04	145.04	6.79*
pH x TXC	1	614.08	307.04	14.36*
Residual	12	256.50	21.38	
Total	23			

Mean = 50.5

Cell Means

pH x T x C (LSD = 7.1)

(2,4,0)	(2,4,2)	(2,24,0)	(2,24,2)	(7,4,0)	(7,4,2)	(7,24,0)	(7,24,2)
49.5	5.5	31	5	86.5	69	69	22

(10,4,0)	(10,4,2)	(10,24,0)	(10,24,2)
87.5	74	69.5	38

* significant at 0.05 level

APPENDIX E

EXTRACTION AND CLEANUP PROCEDURES FOR WASTEWATERS

METHOD OF EXTRACTION FOR NITROSAMINES IN WASTEWATER

1. Measure 1-liter water sample in 1-liter graduated cylinder (pH 7-10).
2. Pour into 2-liter separatory funnel.
3. Extract three (3) times with 60 mL each DCM (2 min. shake).
4. Drain each DCM extract into 250-mL separatory funnel.
5. Add 10-mL 10% HCl and shake for 2 min.
6. Drain DCM (through Na_2SO_4) into Kuderna-Danish (rinse Na_2SO_4 with DCM prior to use).
7. Evaporate 7-8 mL into 70-75° water bath.
8. Evaporate to 1.8-2.0 mL under nitrogen stream.

ALUMINA COLUMN CLEANUP PROCEDURE FOR NITROSAMINES

Prepare alumina by adding 2% water to alumina[1] W200 basic, activity grade Super I.

Use 1 cm ID column chromatography tube with glass frit and Teflon stopcock (Kontes Catalog K420540-0213).

1. Add 12 g of prepared alumina to chromatography tube and tap to settle.
2. Add about 5 mm Na_2SO_4.
3. Add 10 mL of 30% ethyl ether[2] in pentane and drain to top of Na_2SO_4--discard the 1-2 mL eluate.
4. Place sample in 1.8-2.0 mL DCM in tube and drain to top of Na_2SO_4.
5. Wash sample onto column with 2 mL pentane and drain to top of Na_2SO_4.
6. Add 30% ethyl ether in pentane to tube.
7. Discard the first 10 mL collected.
8. Collect the next 60 mL (Fraction I) adding solvent so level is even with Na_2SO_4 after collection of Fraction I.
9. Add 50% ethyl ether in pentane to tube.
10. Collect the next 60 mL fraction (Fraction II).
11. Transfer each fraction to a Kuderna-Danish evaporator.
12. Add 15 mL methyl alcohol to K-D containing Fraction II.
13. Concentrate each fraction to 6-7 mL by placing K-D in 70-75°C water bath.
14. Transfer each fraction to 10 mL volumetric flask.

Fraction I - NDPhA + small amount NDPrA.
Fraction II - NDMA + most of NDPrA + DPhA.

NOTE 1: Obtained from ICN Pharmaceuticals, Inc., Catalog 404571.
NOTE 2: Use ethyl ether with 2% ethyl alcohol as preservative.

FLORISIL COLUMN CLEANUP PROCEDURE FOR NITROSAMINES

Prepare Florisil PR Grade by heating to 130°C overnight or longer.

Use 22 mm ID column chromatography tube with glass frit and Teflon stopcock (Kontes Catalog K420540-0234).

1. Add 22 g of Florisil PR to 22 mm ID chromatography tube and tap to settle.
2. Add about 5 mm Na_2SO_4 to top of column.
3. Add 40 mL of 15% ethyl ether in pentane to tube.[1]
4. Drain until solvent level is even with opt of Na_2SO_4.
5. Add sample in 1.8-2.0 mL DCM to top of column.
6. Drain sample onto column (top of Na_2SO_4).
7. Wash sides of tube with 2 mL pentane.
8. Drain to top of Na_2SO_4.
9. Position sample collector for collection.
10. Add about 85 mL 15% ethyl ether in pentane to chromatography tube.
11. Collect 90 mL adding small amounts of solvent so that solvent level is even with Na_2SO_4. This is Fraction I.[2]
12. Add about 100 mL 5% acetone in ethyl ether to tube and collect 100 mL. This is Fraction II.[3]
13. Transfer each fraction to a Kuderna-Danish evaporator; add 15 mL methyl alcohol to each.
14. Evaporate at 70-75°C to 6-7 mL and transfer to 10 mL volumetric flask.

Fraction I: DPhA (recovery is poor apparently due to degradation on column).
Fraction II: NDMA, NDPrA, and NDPhA

Notes 1. Use ethyl ether with 2% ethyl alcohol as preservative.

2. Fraction I should not have any NDPhA. A peak with a retention time of DPhA is Fration I is due to DPhA <u>not</u> NDPhA.

3. DPhA should not be in Fraction II. A peak with a retention time DPhA in Fraction II results from the degradation of NDPhA to DPhA in the injector. It is not possible, by examining the chromatogram, to determine whether NDPhA or DPhA was injected into the chromatogram.

APPENDIX F

WASTEWATER CHROMATOGRAMS

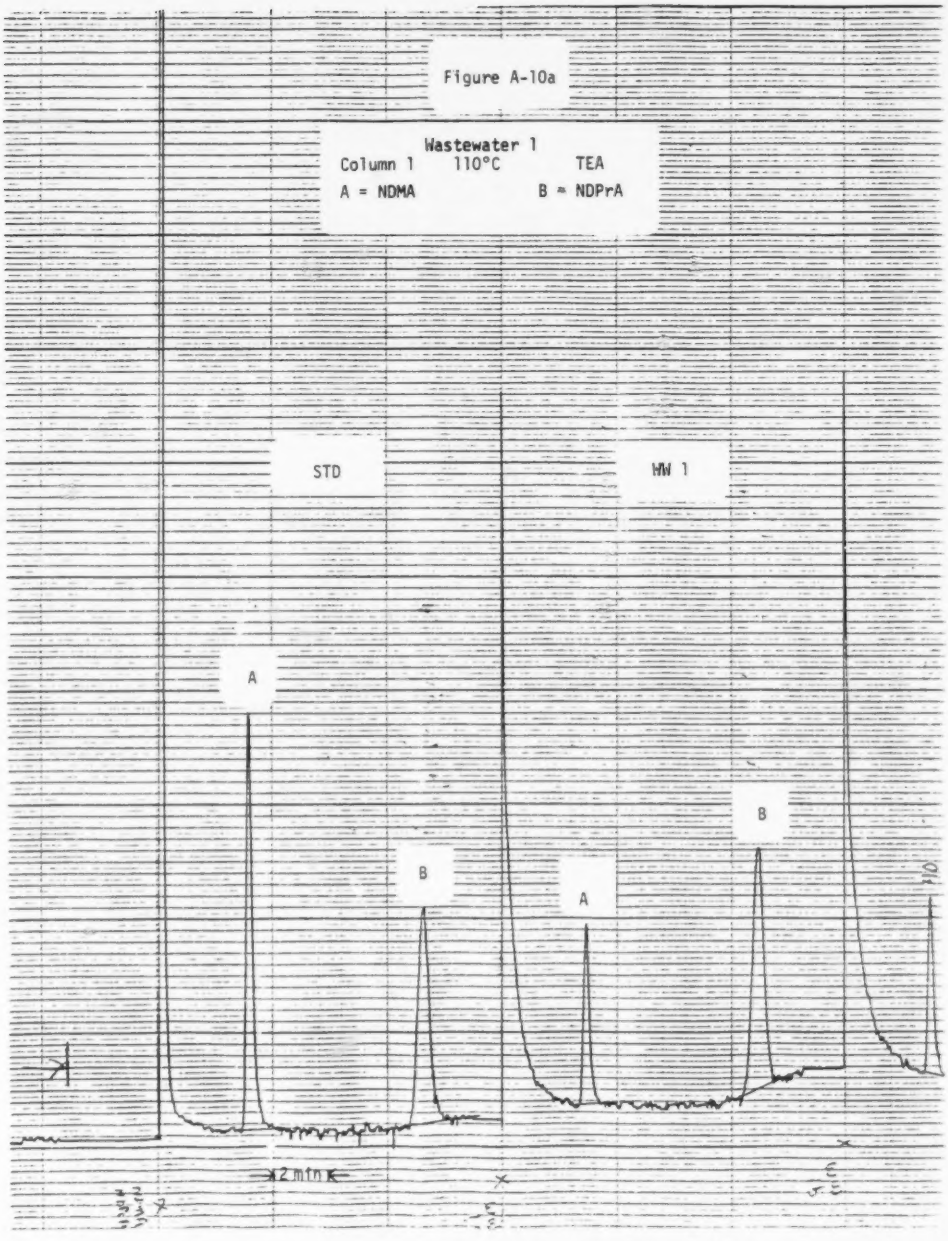


Figure A-10a
Wastewater 1
Column 1 110°C TEA
A = NDMA B = NDPrA
STD
WW 1
A
B
A
B
2 min

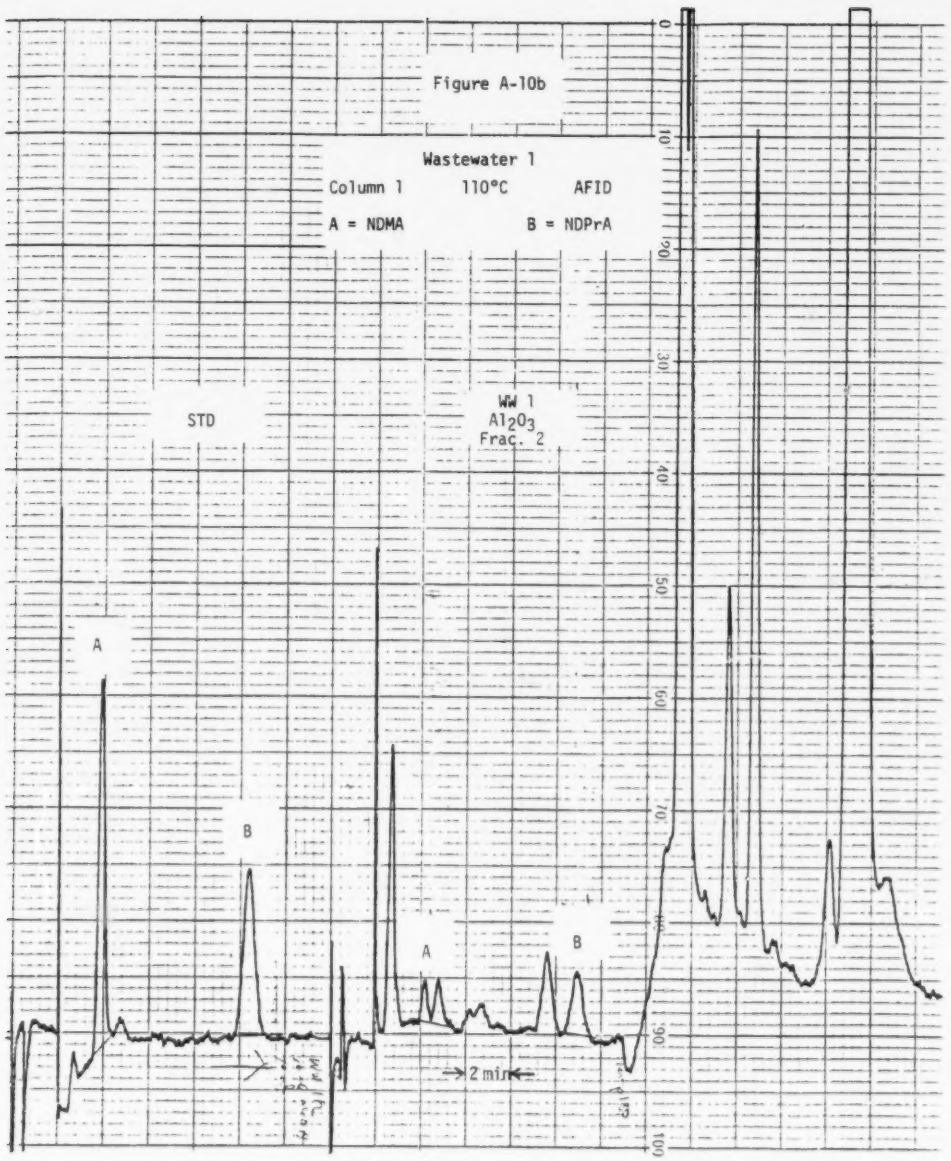


Figure A-10b
Wastewater 1
Column 1 110°C AFID
A = NDMA B = NDPrA
STD
WW 1
Al_2O_3
Frac. 2
A
B
A
B
2 min
0
10
20
30
40
50
60
70
80
90
100

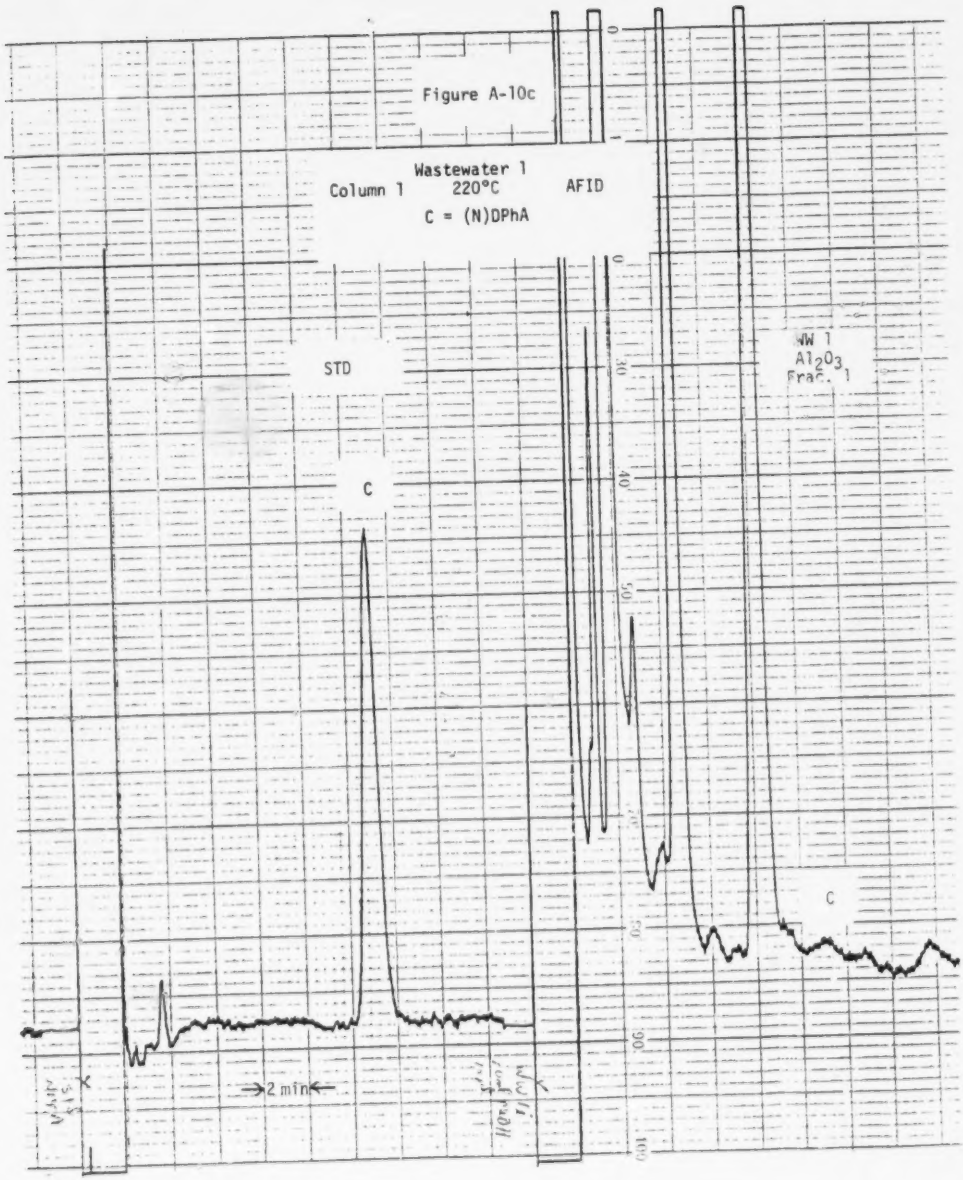


Figure A-10c
Wastewater 1
Column 1
220°C
AFID
C = (N)DPhA
STD
C
WW 1
Al_2O_3
Frac. 1
C
2 min

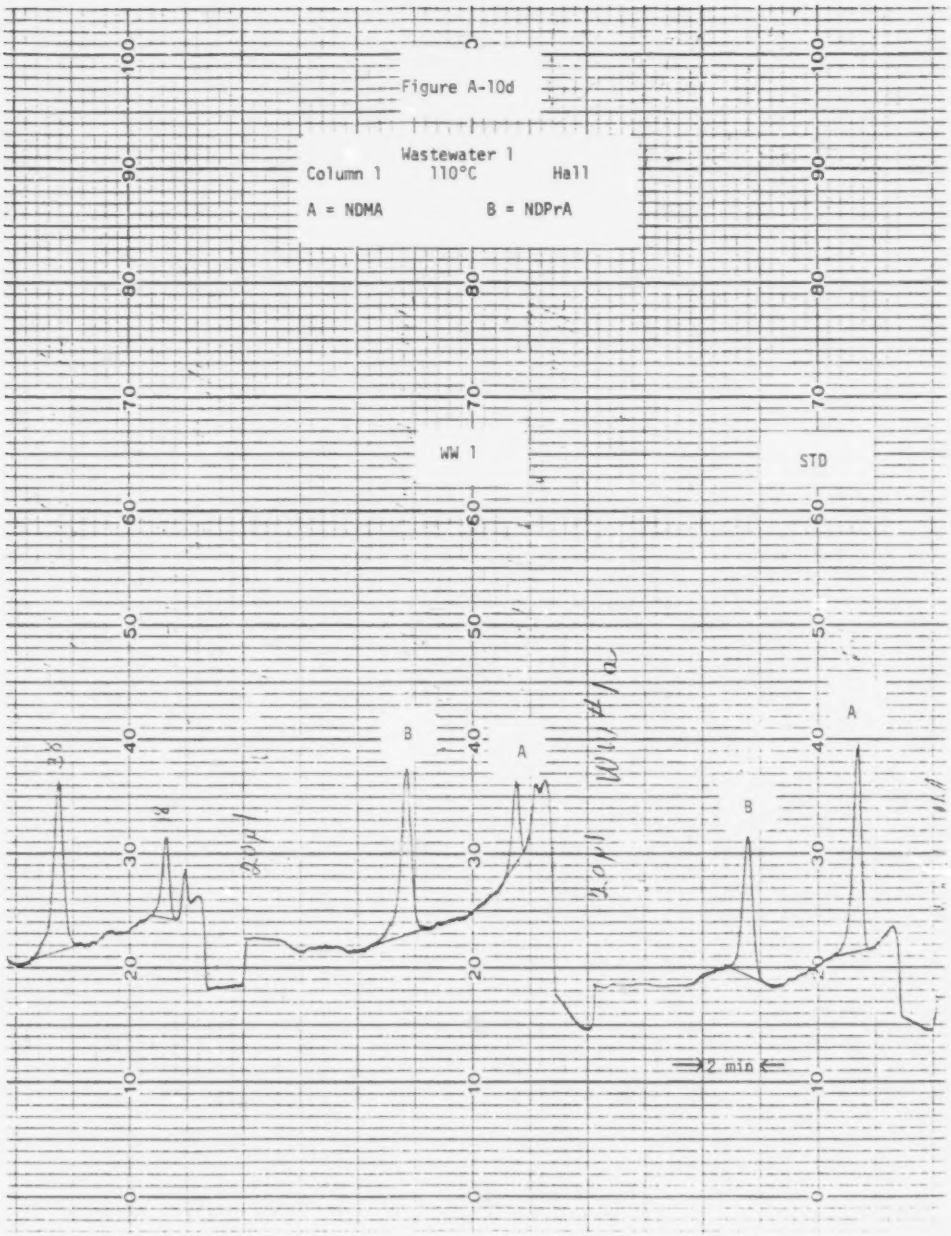


Figure A-10d
Wastewater 1
Column 1 110°C Hall
A = NDMA B = NDPrA
WW 1
STD
B
A
B
A
2 min

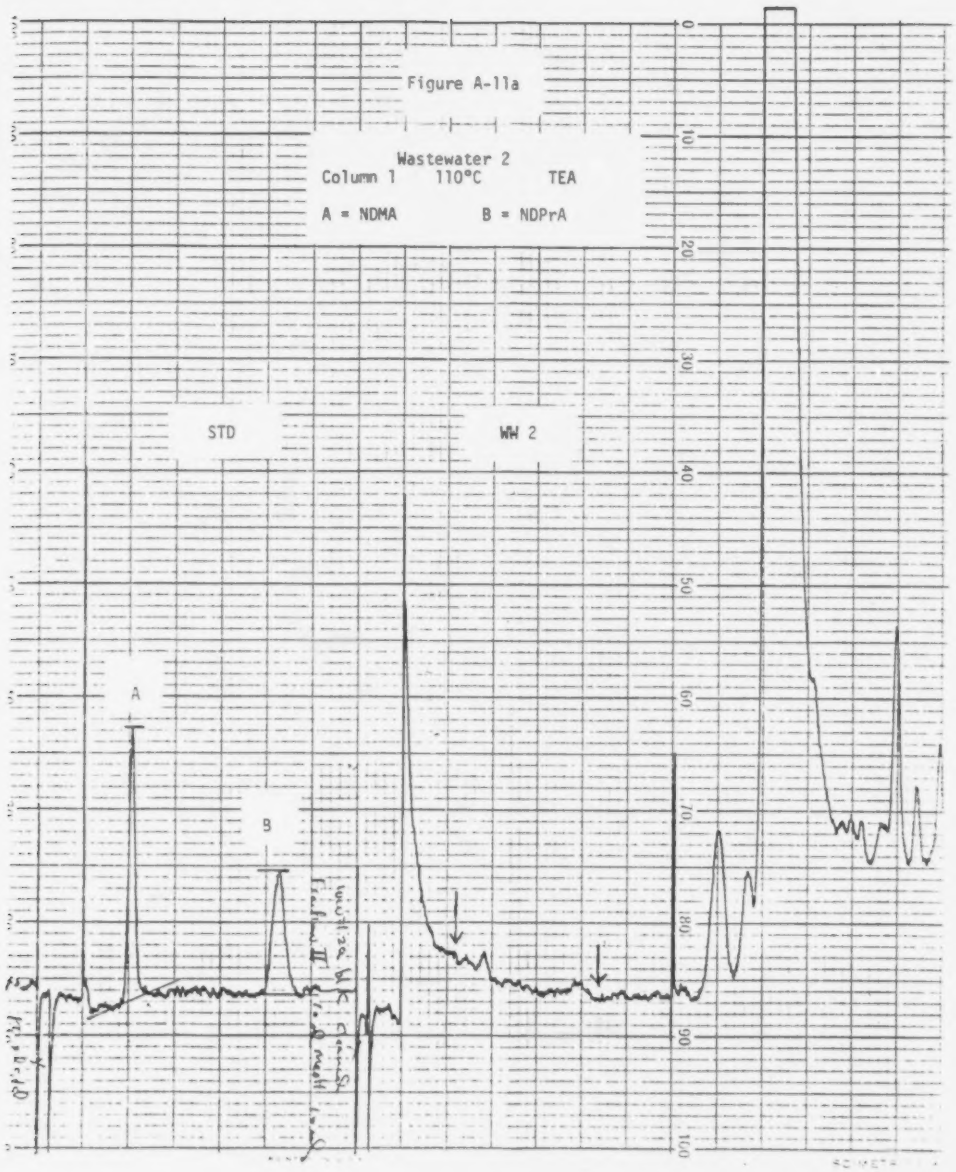


Figure A-11a
Wastewater 2
Column 1 110°C TEA
A = NDMA B = NDPrA
STD
WW 2
A
B
0
10
20
30
40
50
60
70
80
90
100

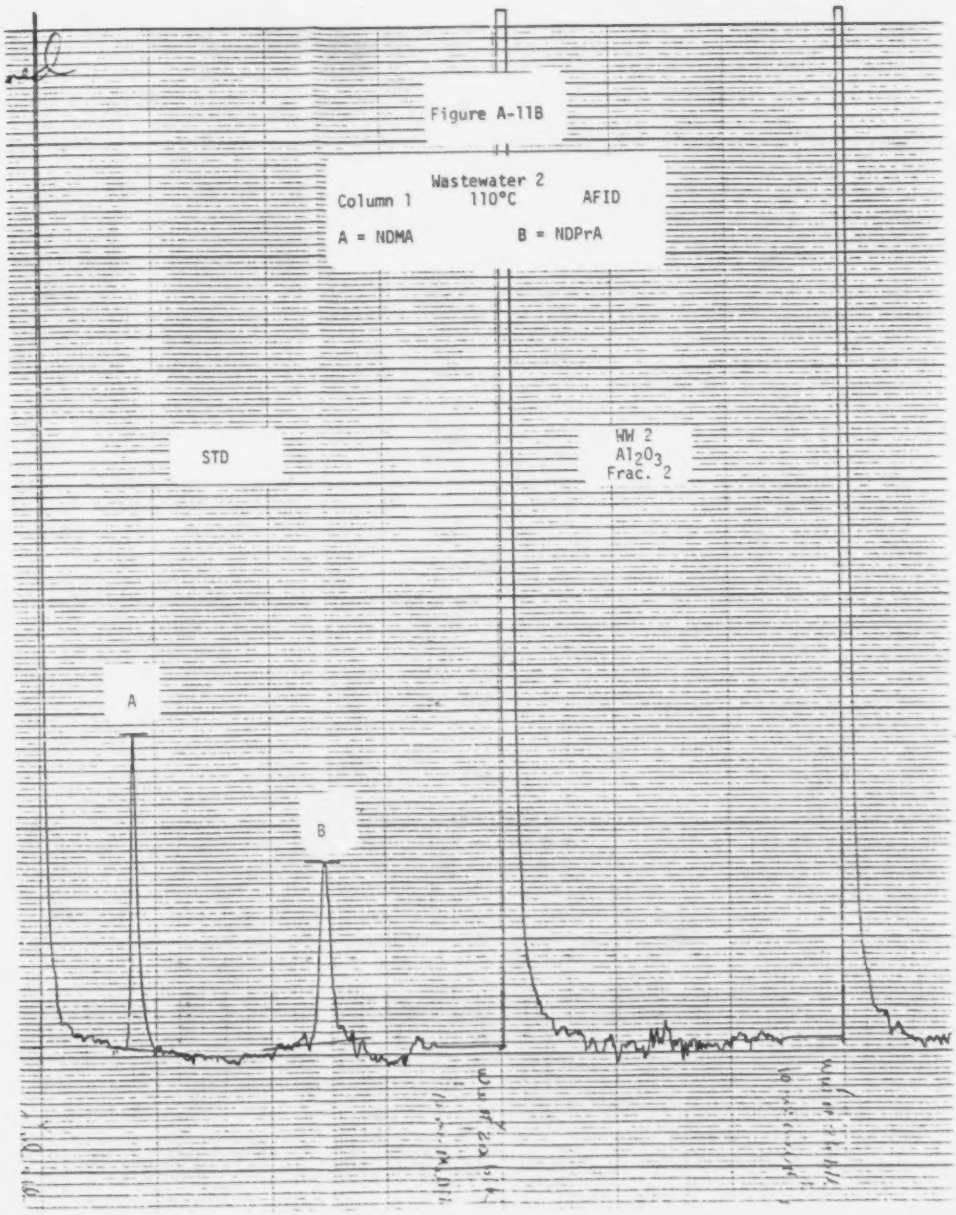


Figure A-11B
Wastewater 2
Column 1
110°C
AFID
A = NDMA
B = NDPrA
STD
WW 2
Al_2O_3
Frac. 2
A
B

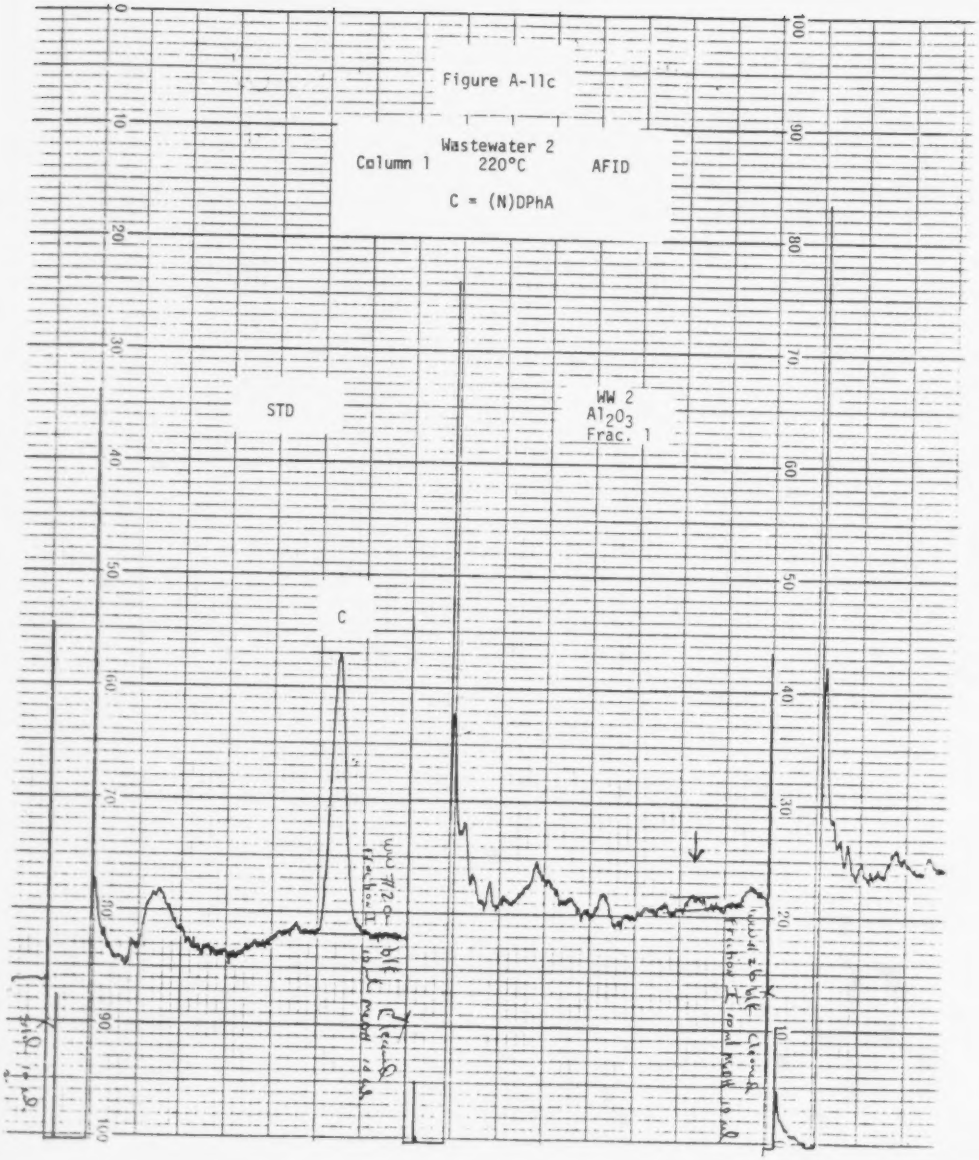


Figure A-11c
Wastewater 2
Column 1 220°C AFID
C = (N)DPhA
STD
C
WW 2
Al_2O_3
Frac. 1

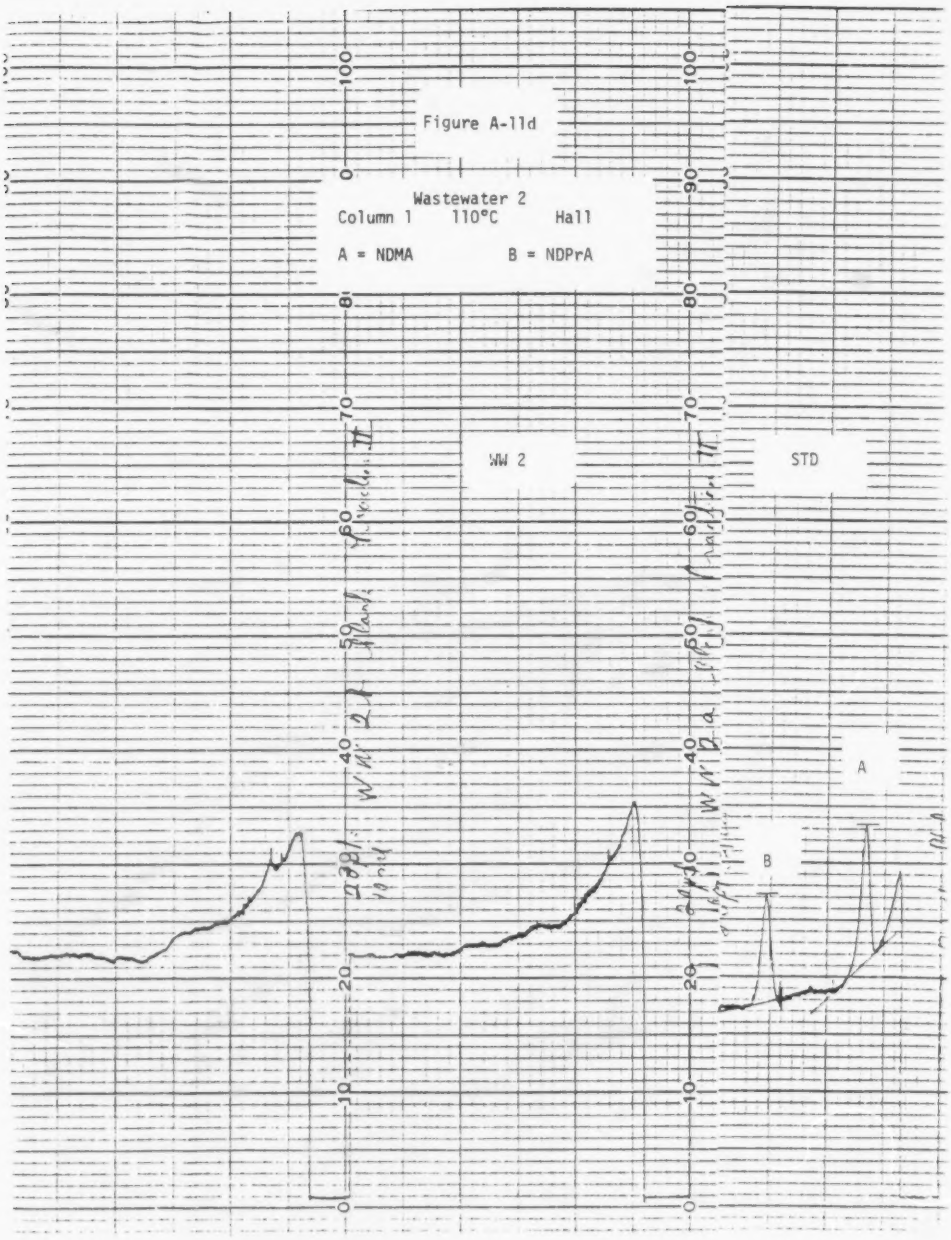


Figure A-11d
Wastewater 2
Column 1 110°C Hall
A = NDMA B = NDPrA
WW 2
STD
A
B

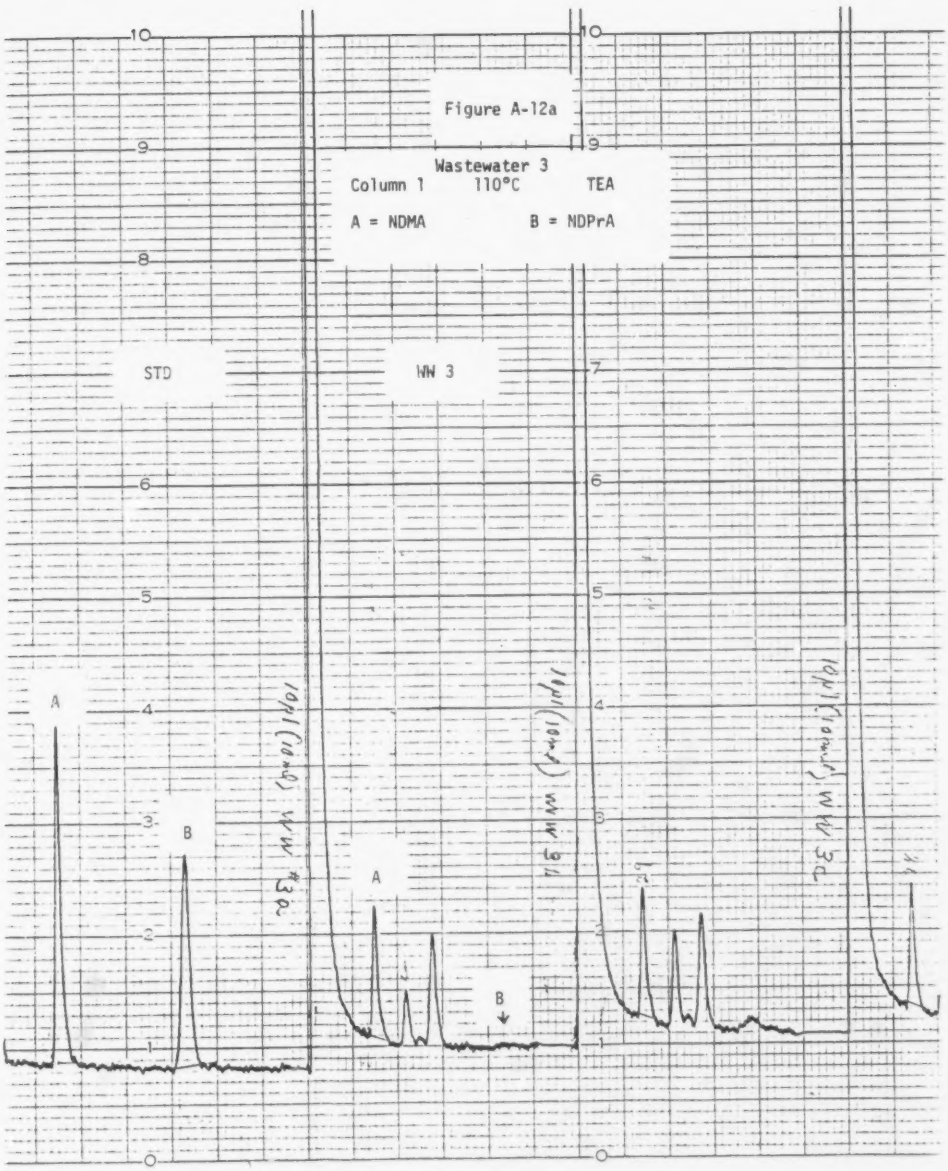


Figure A-12a
Wastewater 3
Column 1 110°C TEA
A = NDMA B = NDPrA
STD
WW 3
A
B
A
B

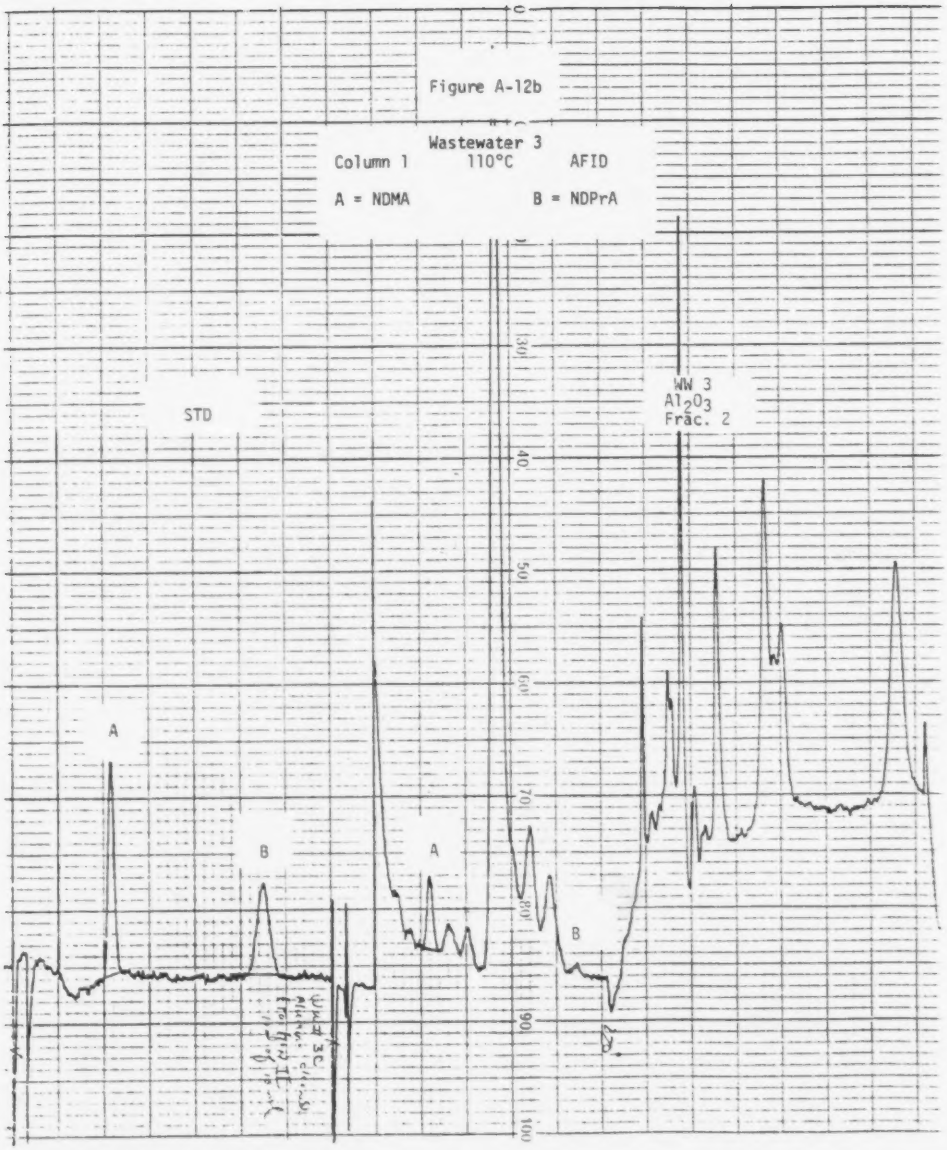


Figure A-12b
Wastewater 3
Column 1 110°C AFID
A = NDMA B = NDPrA
STD
WW 3
Al_2O_3
Frac. 2
A
B
A
B

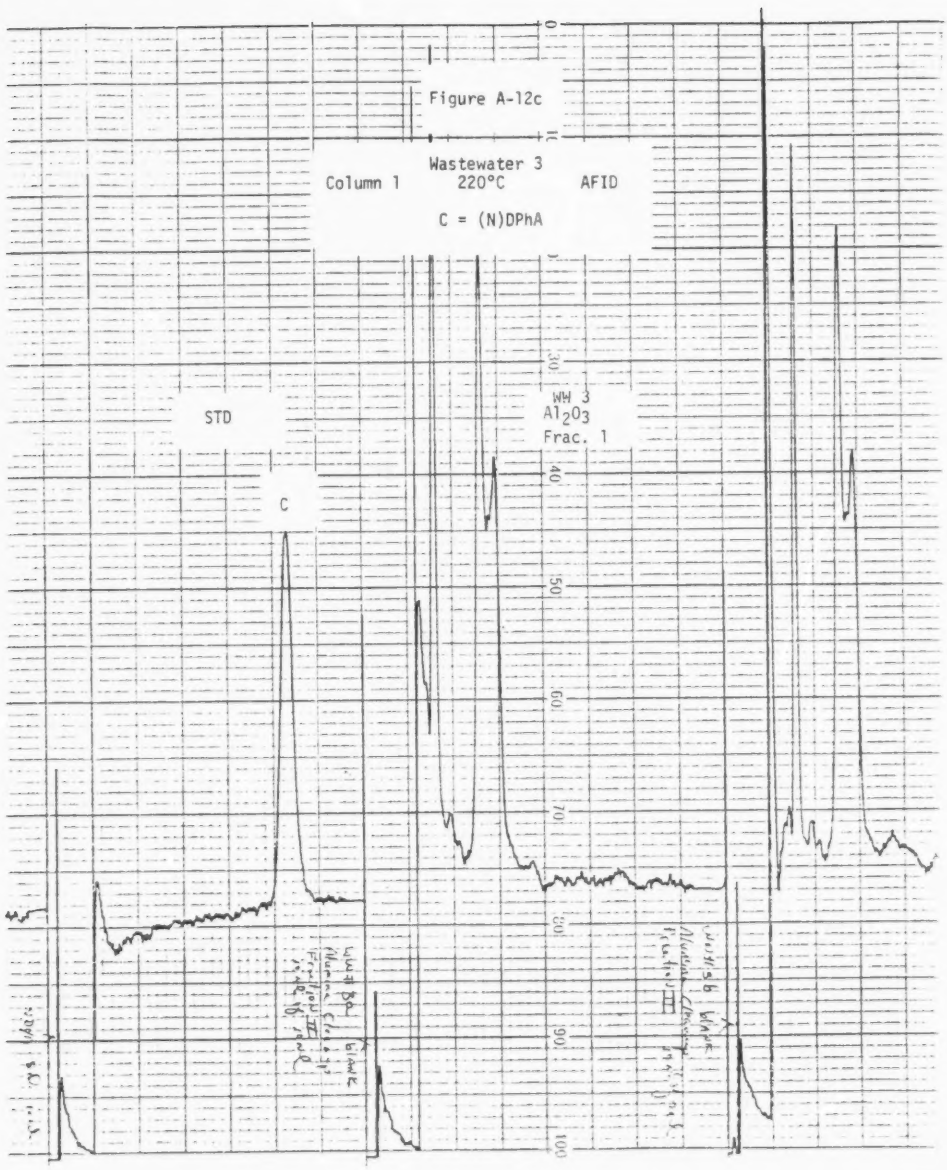


Figure A-12c
Wastewater 3
Column 1
220°C
AFID
C = (N)DPhA
STD
C
WW 3
Al_2O_3
Frac. 1

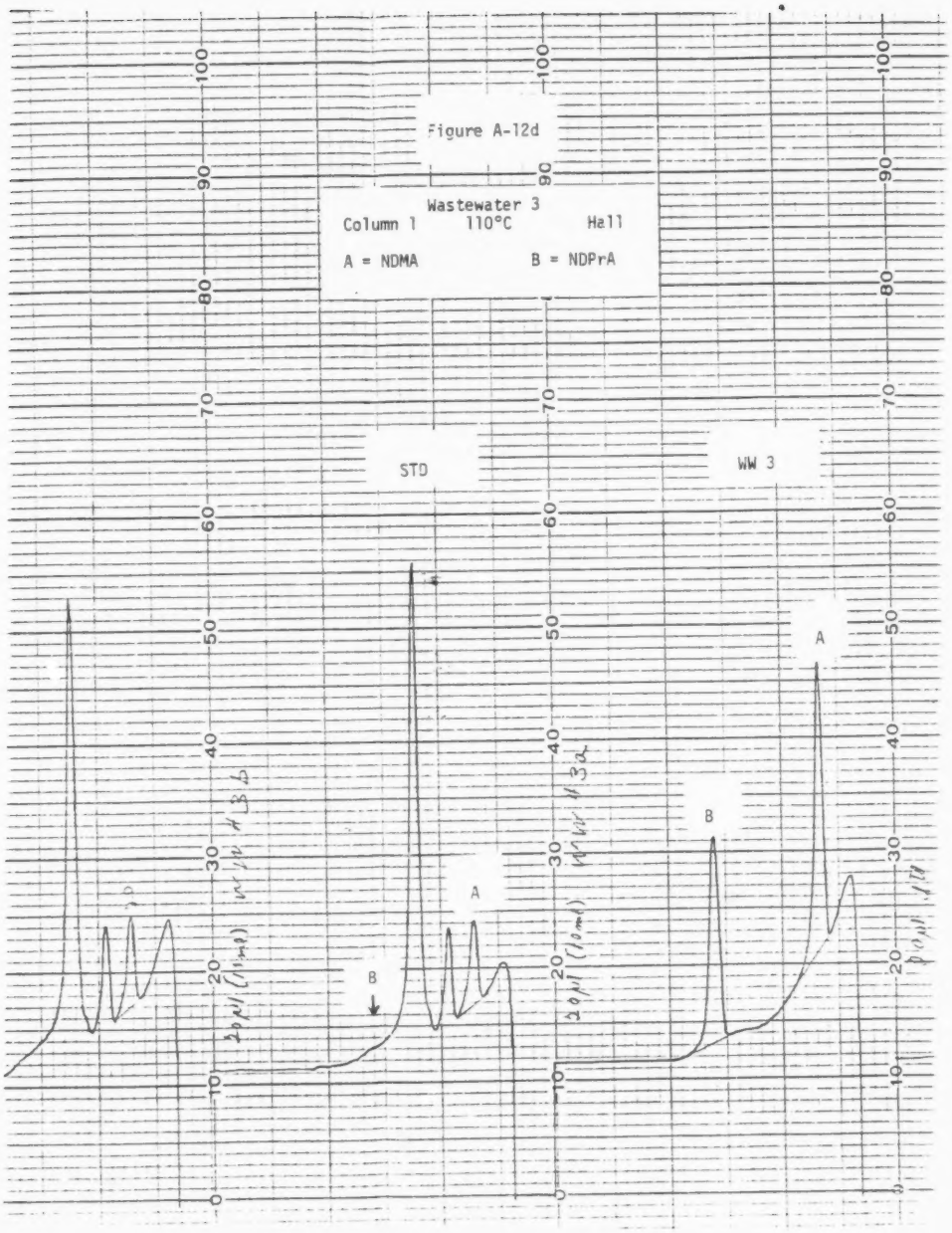


Figure A-12d
Wastewater 3
Column 1 110°C Hall
A = NDMA B = NDPrA
STD
WW 3
A
B
A
B

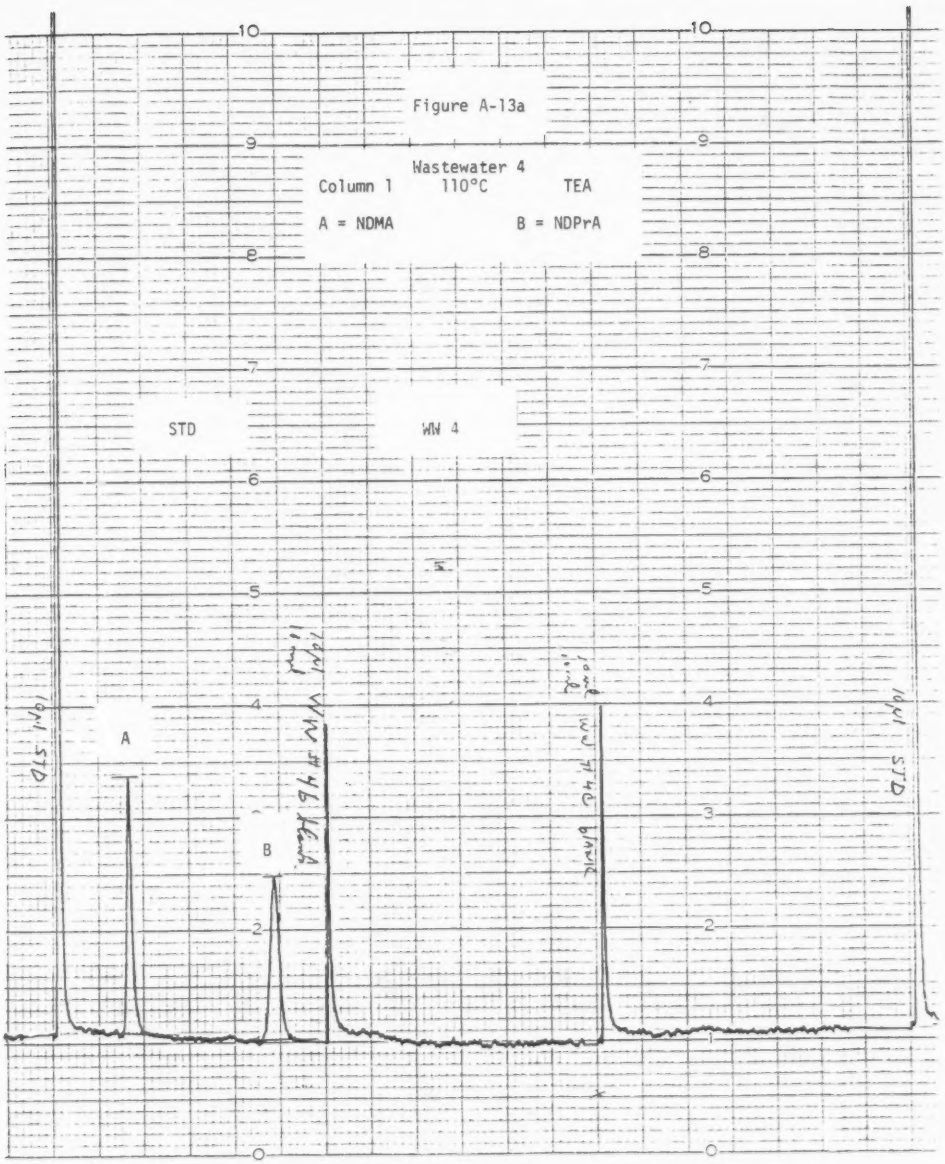


Figure A-13a
Wastewater 4
Column 1
110°C
TEA
A = NDMA
B = NDPrA
STD
WW 4
A
B
10
9
8
7
6
5
4
3
2
1
0

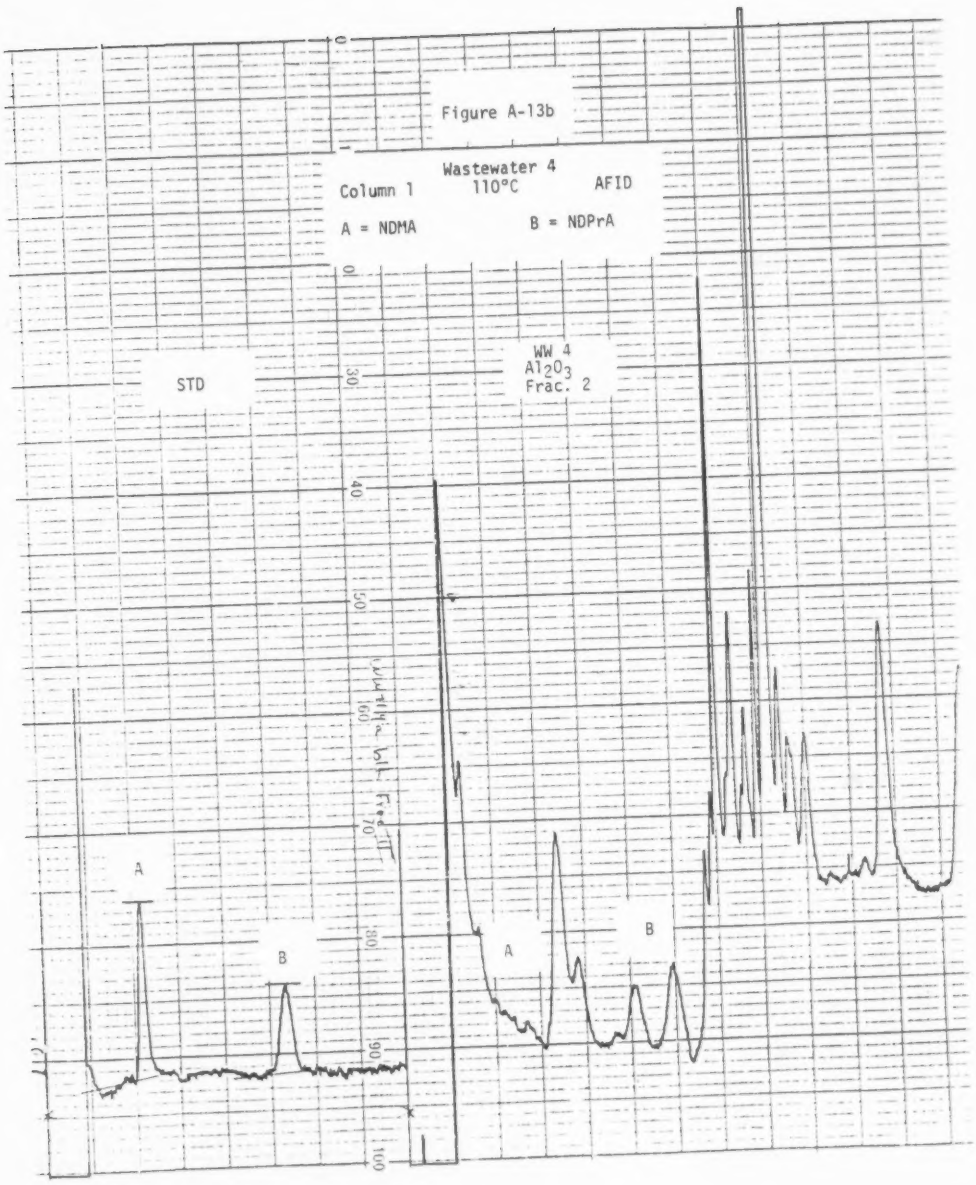


Figure A-13b
Wastewater 4
Column 1
110°C
AFID
A = NDMA
B = NDPrA
STD
WW 4
Al_2O_3
Frac. 2
A
B
A
B

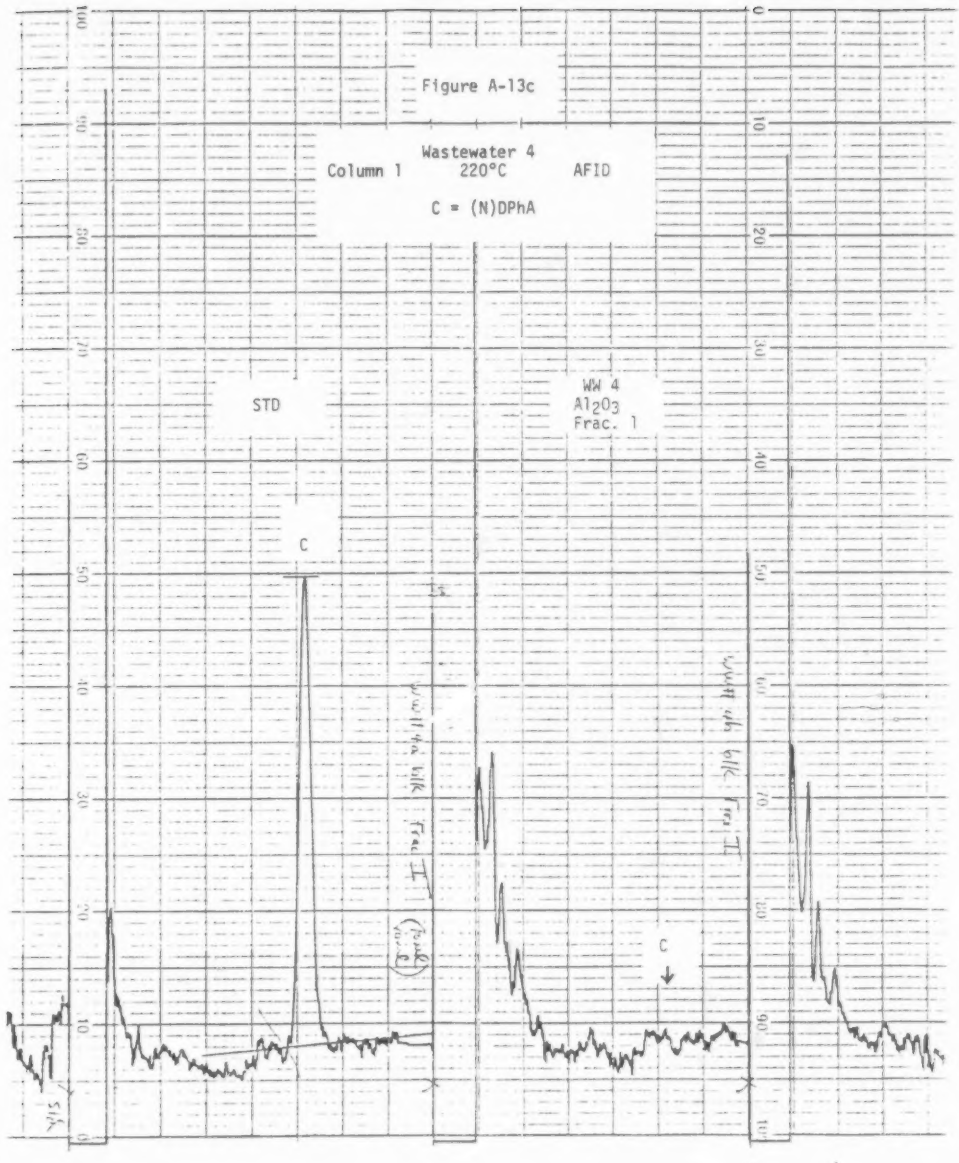


Figure A-13c
Wastewater 4
Column 1
220°C
AFID
C = (N)DPhA
STD
C
WW 4
Al_2O_3
Frac. 1
C

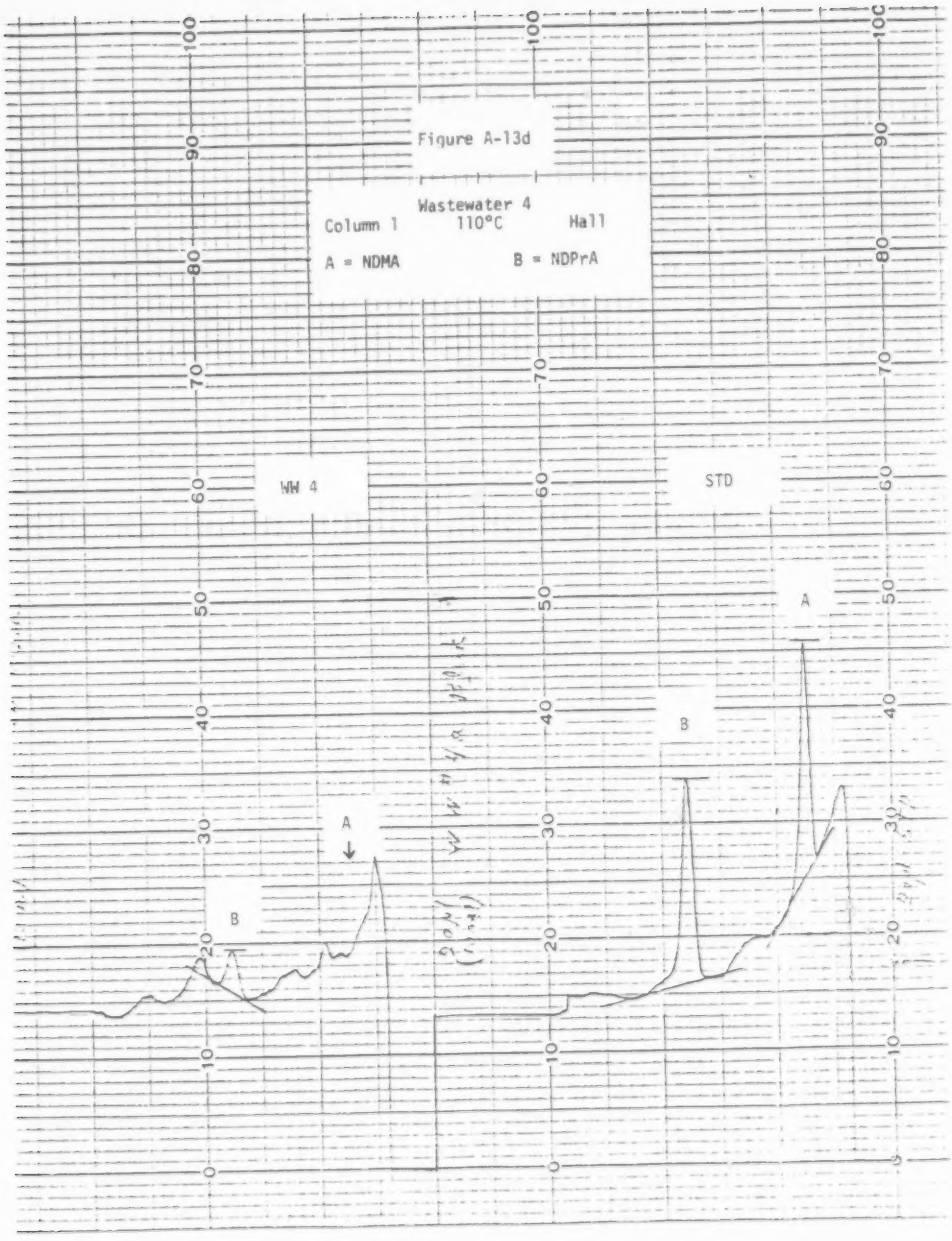


Figure A-13d
Wastewater 4
Column 1
110°C
Hall
A = NDMA
B = NDPrA
WW 4
STD
A
B
A
B

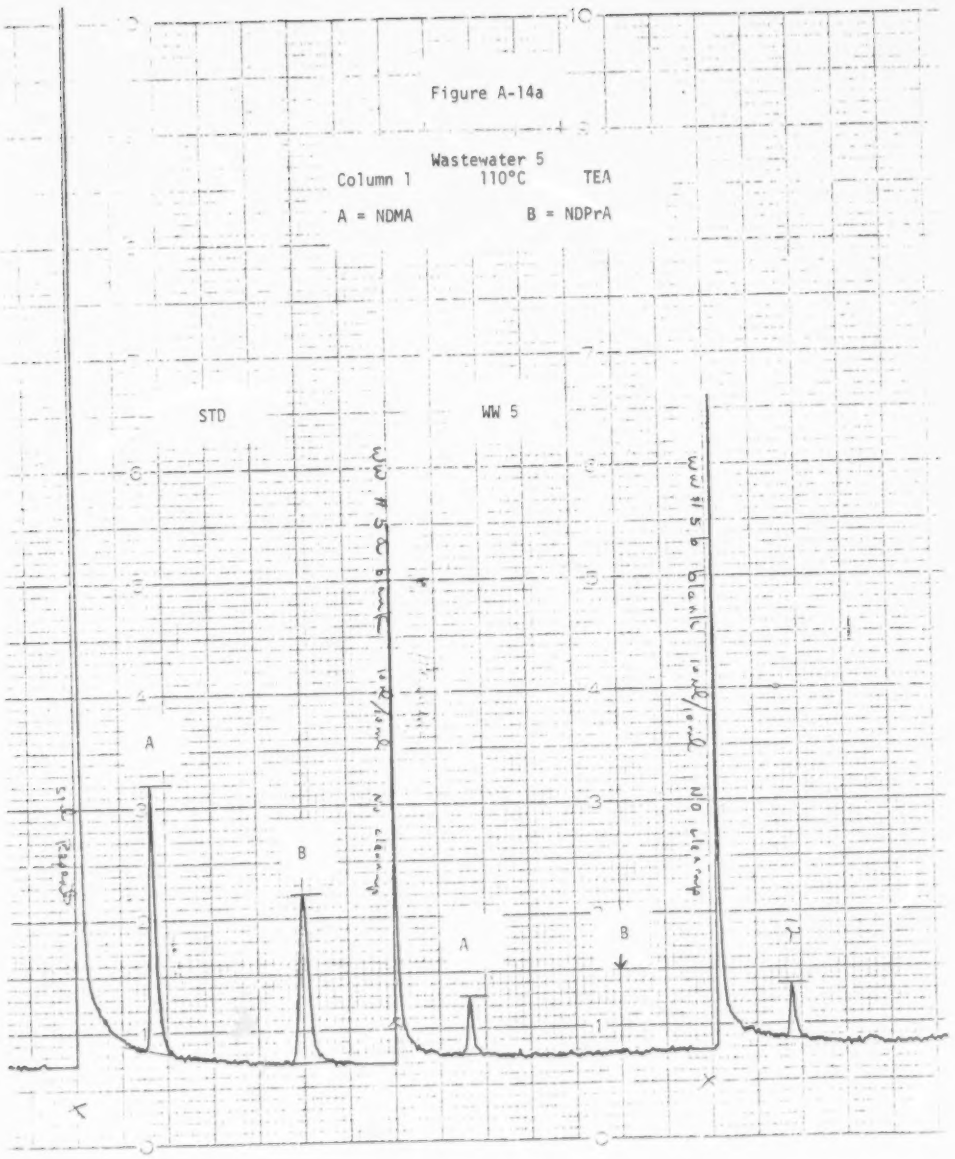
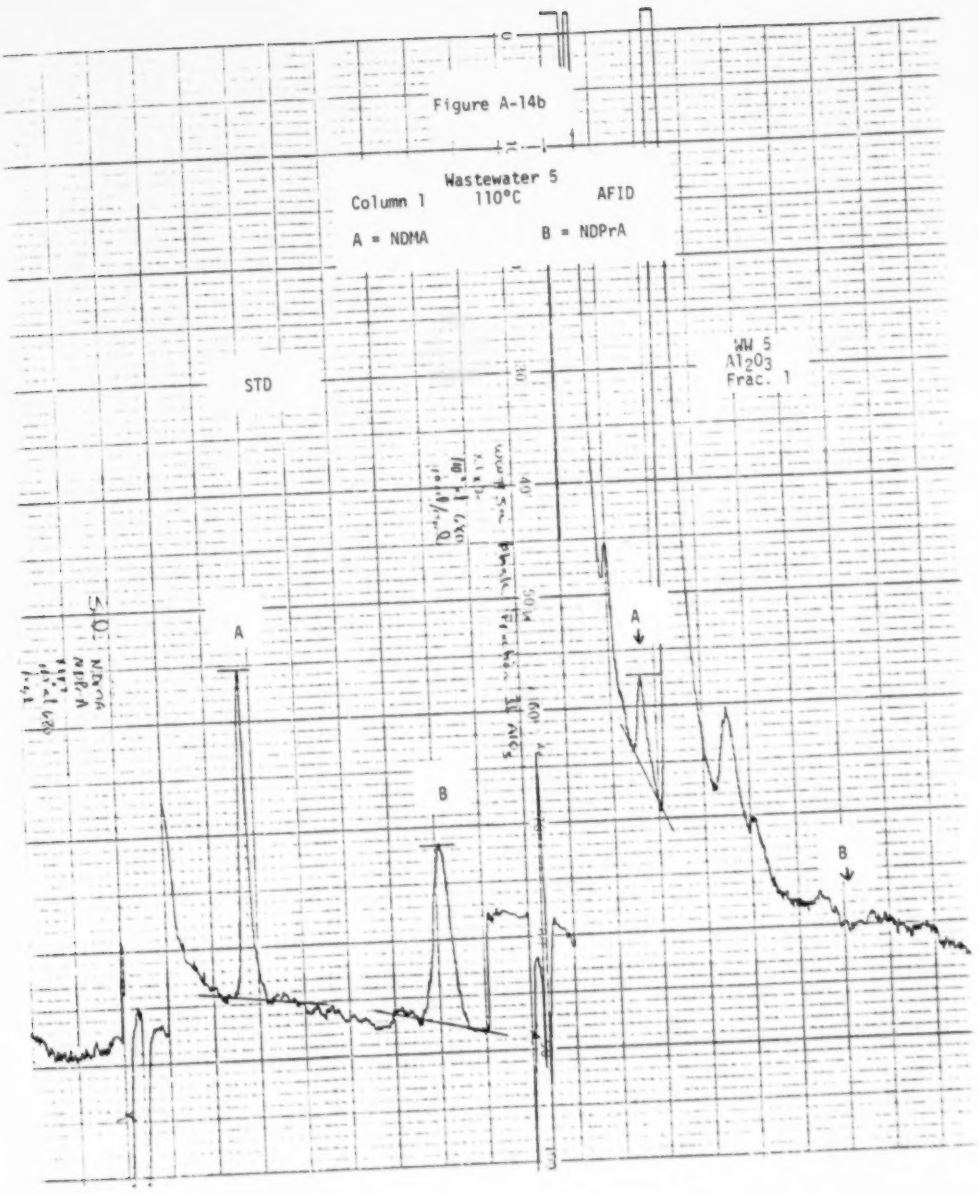


Figure A-14a
Wastewater 5
Column 1
110°C
TEA
A = NDMA
B = NDPrA
STD
WW 5
A
B
A
B

Figure A-14b


Wastewater 5
Column 1
110°C
AFID
A = NDMA
B = NDPrA
STD
WW 5
Al_2O_3
Frac. 1
A
B
A
B

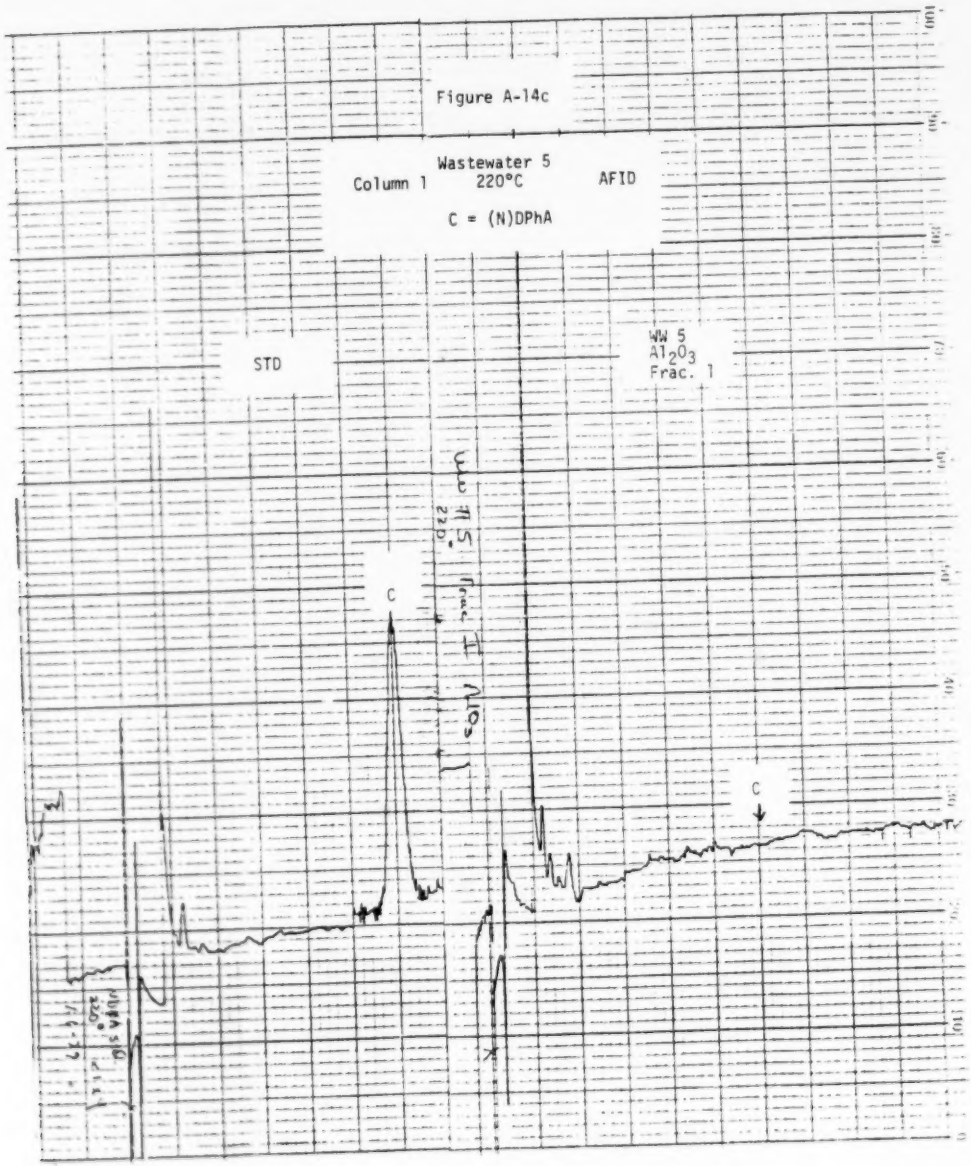


Figure A-14c
Wastewater 5
Column 1
220°C
AFID
C = (N)DPhA
STD
WW 5
Al_2O_3
Frac. 1
C
C

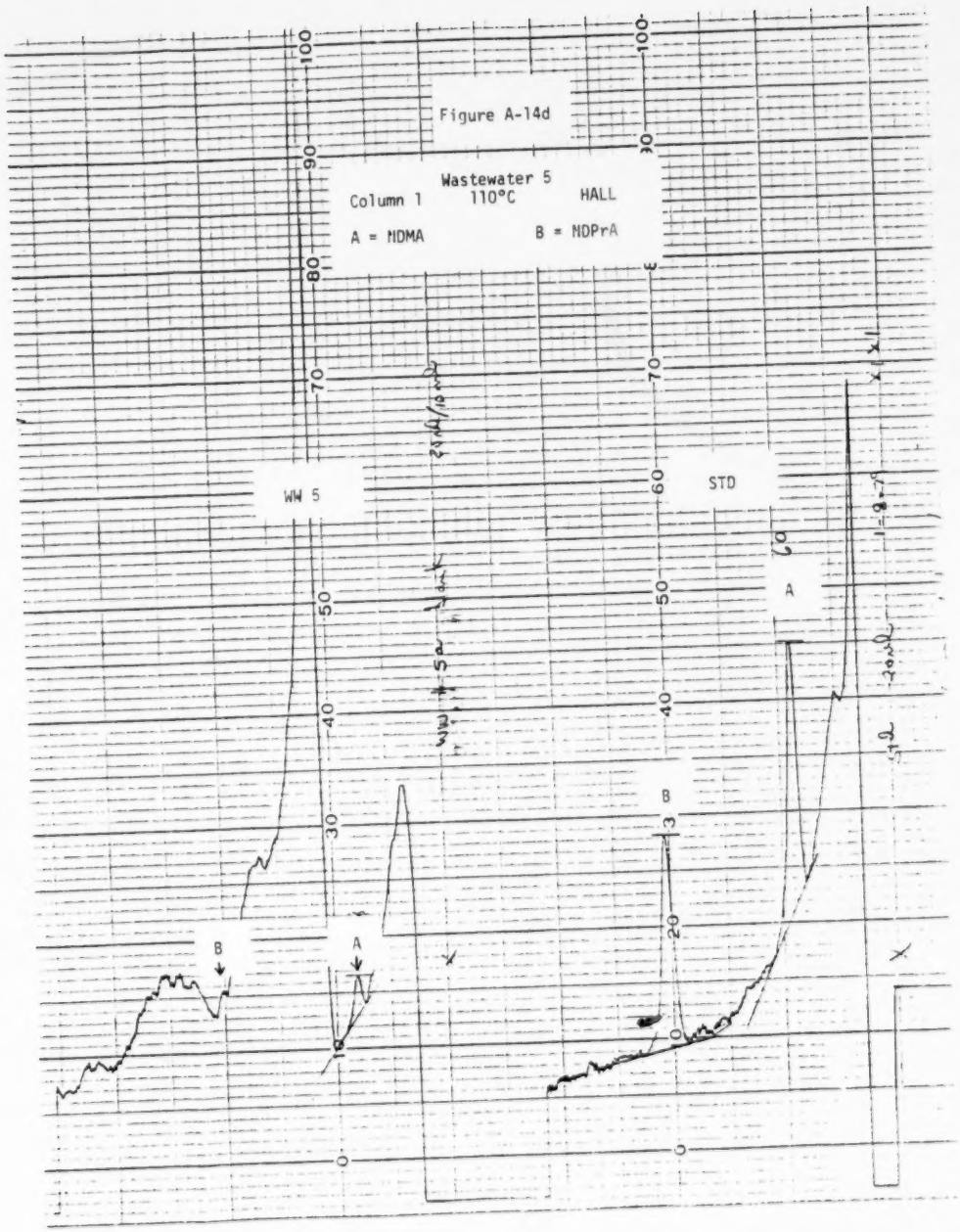


Figure A-14d
Wastewater 5
Column 1 110°C HALL
A = MDMA B = MDPrA
WW 5
STD
A
B
A
B